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CYTOLOGY OF THE TRIBE MADINAE, FAMILY COMPOSITAE

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(WITH ONE HUNDRED AND FOUR FIGURES)

Introduction

The study here reported was begun in the spring of 1931 at the request of Dr. HARVEY MONROE HALL, of the Division of Plant Biology, Carnegie Institution of Washington, who had been occupied for many years previously with a revision of the tribe Madinae of the family Compositae. Dr. HALL had reached the point where he wished to have available information regarding the cytological constitution of certain of the forms whose exact taxonomic disposition had occasioned considerable difficulty. The writer undertook during that year to investigate such forms as were designated by Dr. HALL, plus several additional species which were thought to be deserving of examination. Following Dr. HALL's unfortunate demise early in 1932, the investigations on the Madinae were continued by his associates, Dr. DAVID D. KECK and Dr. J. CLAUSEN. After the peculiar cytological situation in the tribe was revealed they generously offered the writer the opportunity of investigating all the forms which were available during 1932. They have checked over all matters with which the revision might be concerned. The names for genera, sections, and species have been provided by Dr. KECK.

The present paper is obviously not the proper place for the publication of new species nor for the formal notation of other taxonomic changes. Although the arrangement under each of the various genera

will reveal to a certain extent the eventual disposition of its individual species, what has thus been indicated should not be understood to be final, for much work yet remains to be performed before such action becomes possible. In order that the final monograph and what is presented in this paper may be co-ordinated, the field numbers and localities of most of the forms studied have been cited; the few exceptions are in the cases of certain species, such as *Hemizonia angustifolia*, in which such a large number of plants were examined that space cannot be spared for their citation.

Materials

By far the greater proportion of the plants upon which this study is based were originally collected by Dr. HALL and grown in the garden of the Carnegie Institution on the Stanford University campus. All numbers in the 12th and 13th thousands cited herein are Dr. HALL'S collection numbers. The species secured by Dr. KECK bear his own collection numbers preceded by his surname (for example, *Keck 1323*). Those collected by Mr. WM. M. HEUSI of the Institution staff, who was in charge of the cultivation of all the garden plants, are similarly denoted (for example, *Heusi 110*). Vouchers of all plants examined cytologically have been preserved. The writer's own collections of material were made directly from the plants in the field. At the time of obtaining the material, vouchers were also made and given the writer's collection numbers (for example, *DAJ 690*). All such specimens were examined and identified by Dr. KECK.

It has been impossible to obtain either seeds or suitable cytological material of the two Hawaiian representatives of the Madinae, *Argyroxiphium* DC. and *Wilkesia* Gray. Representatives of all the Californian and Mexican genera with the exception of the monotypic genus *Holocarpha* Greene have been available. *Blepharipappus scaber* Hooker, placed in the Madinae by JEPSON (3), is not considered to belong in the tribe, and has not been examined cytologically.

Except as otherwise indicated, all the localities cited are within the state of California. As a rule the county is not given save when confusion might result, or when there are two distinct localities bearing the same name.

In this paper the genera have been arranged alphabetically, and the sections and species are similarly disposed under each genus. No attempt has been made at this time to evolve a phylogenetic scheme for the tribe.

Methods

In the earlier phases of the work, root tips were obtained by placing young seedlings in Knop's solution; but in the later stages, because of the large number of plants to be examined, the individual plants were simply knocked out of the pots in which they were growing and root tips obtained from the mass of roots to be found between the soil and the pot. The water-culture tips were more favorable from the technical standpoint, in that the chromosome plates were more numerous and the elements more evenly spaced.

In 1931, Taylor's modified chrom-acetic plus 1% of either maltose or urea was principally used as killing fluid for the tips, but in 1932, Nawaschin's fluid was substituted throughout. It was soon found that none of the staining methods in common use was satisfactory, consequently it became necessary to devise one more reliable (4). As finally worked out, the method has been improved to the extent of substituting methyl violet 2B for "gentian violet" so-called, and using erythrosin B both as counterstain and to differentiate the violet a little further (both stains were from Coleman and Bell). A clear, sharp stain of optimum intensity invariably resulted. The buds were removed from the plant and the upper portion of the capitulum sliced off. They were then dropped into Carnoy's fluid, whence, after about 8 minutes, they were transferred to Nawaschin's fluid. The fixation was ordinarily excellent, but Nawaschin's fluid occasionally proved erratic in its action. The buds were stained exclusively with methyl violet 2B alone. When possible, and in general, both haploid and diploid counts were made on the same plant; but the drawings of the somatic and meiotic chromosomal complements in any particular form are not necessarily from the same plant.

The preparations were examined and the drawings prepared in the cytological laboratory of the Carnegie Institution, using a Zeiss binocular microscope equipped with a Leitz achromatic-aplanatic condenser, aperture 1.4; Zeiss objective ×120, aperture 1.3, and

Mobimi oculars ×20, giving a bench-level magnification of approximately 4400. "Parallux" parallel illumination was employed.

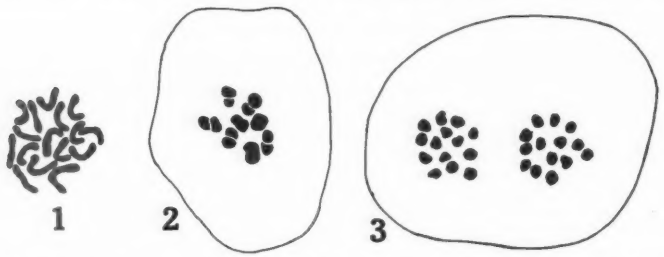
Previous work on the Madinae

There exists only one report of earlier work on the cytology of the Madinae, that of MANN (BABCOCK and HALL 1). This was at best not much more than an exploratory examination, and the preparations employed were of a temporary character. MANN reported finding 12n chromosomes in each of three subspecies of *Hemizonia congesta*, namely, *typica*, *lutescens*, and *luzulaefolia* and 10n chromosomes in *Hemizonia corymbosa* (=*H. angustifolia*). As will be noted later, the counts for *H. angustifolia* have been amply confirmed, but those for the subspecies of *H. congesta* are apparently erroneous.

Observations

ACHYRACHAENA Schauer

This monotypic genus only superficially resembles the other Madinae. The species, *A. mollis* Schauer, an annual, proved very



FIGS. 1–3.—*Achyrachaena mollis:* fig. 1, SM* (2n-16); fig. 2, MI (n-8). *Hemizonia*, section Blepharizonia. *H. plumosa:* fig. 3, MII (n-14). ×2200.

* The following abbreviations are employed in the descriptions of figures: SM, somatic metaphase; MI, first meiotic metaphase; MII, second meiotic metaphase; AI, first meiotic anaphase.

difficult to study cytologically. The root tips are poorly constructed and satisfactory figures are rare; it is also hard to find clear, well stained plates at either the first or the second metaphase of the meiotic divisions. The material principally studied was *13058*, from Priest Valley, supplemented by examination of material collected by the writer on the Stanford campus and on Mt. Hamilton. The haploid number is 8, the diploid 16 (figs. 1, 2). The somatic chromo-

somes are small, curved, and so crowded together that few do not overlap. For this reason it is difficult to make exact computations and the number occasionally appeared to be 17 or 18 rather than 16. Trabants ordinarily could not be seen.

Hemizonella Gray

The genus consists of but one species, *H. minima* Gray, a low, montane annual. Only a single plant, which originally came from Mather, was available. Both the root tips and the few buds secured were very unsatisfactory, and it was not possible to make accurate counts in either. Several metaphase plates showed approximately 26 somatic chromosomes. In the microsporocytes, no first or second metaphase plates were encountered. In each of three interphases, however, 13 chromatic bodies were definitely countable.

Hemizonia DeCandolle

The genus is readily separable into six sections which are fairly consistent systematically but not always so cytologically. The sections include: Blepharizonia, Calycadenia, Centromadia, Euhemizonia, Fruticosi, and Hartmannia (in part). All the sections contain only annual plants, with the exception of the Fruticosi, which are perennials.

Section Blepharizonia Gray

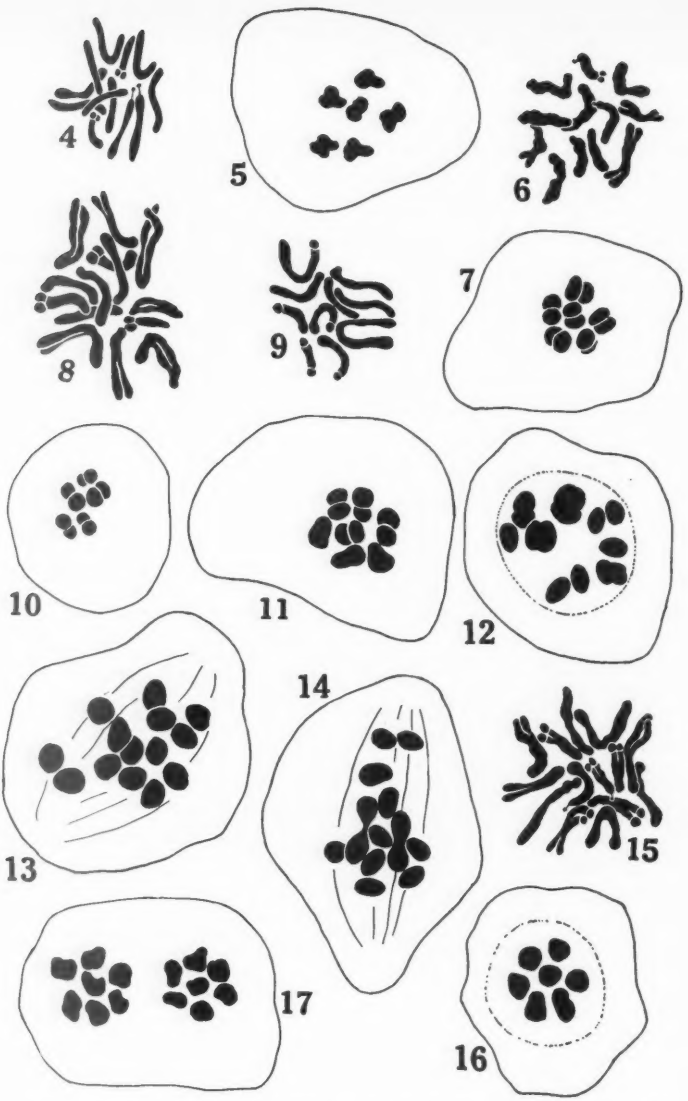
The section contains only the following:

H. plumosa (Kell.) Gray. n-14.—A single garden plant provided buds in which the haploid number was best counted at the second metaphase (fig. 3).

Section Calycadenia Gray

The section consists essentially of the genus *Calycadenia* of DeCandolle and contains perhaps twelve species, of which six have been subjected to cytological examination.

H. bicolor Jepson. n-6, 2n-12.—Four collections were studied; they proved to be identical in all respects cytologically. The somatic chromosomes are noteworthy for their length as compared with their breadth, among the Madinae. Two chromosomes appear to be medianly and two submedianly constricted, but the fixation (chrom-acetic) was apparently not good enough to bring out all the morpho-



FIGS. 4–17.—*Hemizonia* section Calycadenia. *H. bicolor:* fig. 4, SM (2n-12); fig. 5, MI (n-6). *H. ciliosa:* fig. 6, SM (2n-12); fig. 7, MI (n-6). *H. mollis:* fig. 8, SM (2n-14). *H. pauciflora:* fig. 9, SM (2n-10); fig. 10, MI (n-5). *H. truncata:* fig. 11, MI, normal with 7 bivalents; fig. 12, late diaphase with 4 bivalents and 6 univalents; fig. 13, AI with 14 univalents; fig. 14, AI with 2 bivalents and 10 univalents. *H. truncata* ssp. *"scabrella":* fig. 15, SM (2n-14); fig. 16, late diaphase (n-7). *H. villosa:* fig. 17, MII (n-7). ×2200.

logical characteristics (fig. 4). Two trabants are always prominently to be seen. The meiotic chromosomes are somewhat irregular at the first metaphase (fig. 5); at an early stage in the second metaphase there is evidence of secondary association.

H. ciliosa Jepson. n-6, 2n-12.—Four groups of plants were available for examination. As in *H. bicolor*, there is one pair with median and another with submedian constrictions, while the remaining chromosomes appear to lack such characters. The trabants are very tiny and on a short, stout, terminally constricted portion of the chromosome (fig. 6), whereas those in *H. bicolor* are on long threads. Meiotic behavior is regular (fig. 7).

H. mollis Gray. n-7, 2n-14.—Four sets of plants were examined, but all proved to be cytologically identical. The somatic chromosomes of this species are large, and all save possibly one pair have definite submedian constrictions (fig. 8). In most of the chromosomes the longitudinal split appears in late prophase. Trabants are apparently not of regular occurrence.

H. pauciflora Gray. n-5, 2n-10.—This species was studied during 1931, only one plant being available, *12936*, which came originally from near Stonyford, Colusa County. Fixation of the somatic chromosomes was not perfect enough to reveal minute details of the morphology, but it is nevertheless clear that the chromosomes may be grouped according to length into the following pairs: one long U-shaped, one medium long, two short, and one still shorter (fig. 9). These differences are perpetuated in the sizes of the meiotic chromosomes (fig. 10), as the latter include the following sizes: one large, one medium large, two small, and one slightly smaller.

H. truncata Gray. n-7, 2n-14.—Four groups of plants were examined: *12860* from north of Plymouth, Amador County, *12862* from west of Placerville, *Heusi 107* from north of Yountville, and *13238* from hills back of Post's, Monterey County. The somatic divisions were difficult to study; there were practically no clear, flat metaphase plates to be found in the only group of which root tips were studied (*13238*). The diploid number, at any rate, is certainly 14. In one or two instances a chromosome was observed to have been split transversely into two equal portions, but there is no real evidence that fragmentation occurs in this species.

In *12860* (fig. 11) and *12862* meiosis seemed to be perfectly regular, but it was markedly abnormal in *Heusi 107*. In some microsporocytes all the chromosomes existed as univalents, while the following assortments of bivalents and univalents were observed in other mother cells: 2_{II}-10_{I}, 3_{II}-8_{I}, 4_{II}-6_{I}, and 5_{II}-4_{I}. It will be noticed that in each case the total number comes to seven pairs. Some of these configurations are illustrated (figs. 12-14).

H. truncata ssp. "*scabrella*." This subspecies is the equivalent of *H. scabrella* Drew. n-7, 2n-14.—The somatic chromosomes are clearer than in the species, but it is still difficult to diagnose their morphology. Two are invariably bent sharply at the center, but it is not known whether constrictions actually occur there. Others are submedianly to subterminally constricted. The two terminally constricted chromosomes carrying the very small trabants are distinctly the shortest in the entire complement (fig. 15). The meiotic chromosomes seem to be perfectly regular in their entire behavior (fig. 16).

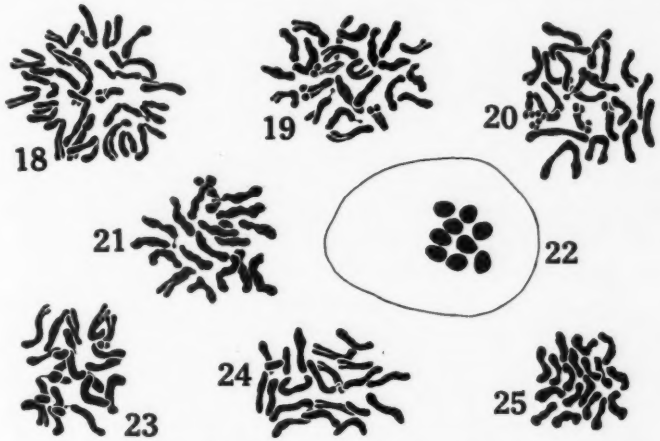
H. villosa Jepson. n-7, 2n-14.—The one group of plants examined, *13157*, was originally secured in Jolon Valley. The somatic chromosomes are rather large, but too interlaced for illustrative purposes. Two terminally constricted chromosomes carry prominent trabants. The meiotic chromosomes at the first metaphase lie too close together, but those at the second metaphase are clearly separated (fig. 17). No morphological differences were observable at either stage or in the somatic mitoses.

Section "CENTROMADIA" (Greene)

The section is equivalent to the genus *Centromadia*, as proposed by GREENE, and includes a homogeneous group of very spinescent species. As most commonly recognized up to the present time, the former genus contained two species, *C. fitchii* (Gray) Greene and *C. pungens* (T. & G.) Greene, the latter including two varieties. *C. fitchii* has unfortunately been unavailable, but a number of forms of *C. pungens* have been examined. From the cytological standpoint, the reduction of *C. maritima* Greene to synonymy under *C. pungens* appears to be justified, although in the present paper it is accorded separate treatment. However, neither of the two varieties, *congdoni*

and *parryi* (3), can be retained as such, but are being restored to their original rank as distinct species, because of the unexpected discrepancy in chromosome numbers.

H. congdoni Rob. & Greenm. 2n–24.—The four groups of plants studied and the localities at which they were originally collected were: *13190* from 4 miles southwest of Salinas, *13193* from south of Salinas, *13273* at Chualar, and *13274* from south of Castroville. These localities are all within the lower part of the Salinas Valley,



FIGS. 18–25.—*Hemizonia* section Centromadia. *H. congdoni:* fig. 18, SM (2n-24) from *13273*, and fig. 19, from *13190*. *H. parryi:* fig. 20, SM (2n-24). *H. pungens:* fig. 21, SM (2n-24); fig. 22, MI (n-9). *H. pungens* ssp. *"maritima":* fig. 23, SM (2n-19 ? with 3 pieces) and fig. 24, SM (2n-21), both from *13261;* fig. 25, SM (2n-18). ×2200.

and incidentally within the range of *H. pungens*. In the somatic complements, *H. congdoni* possesses two long submedianly constricted chromosomes, exactly as in *H. parryi;* in the first species they are always in the center of the plate, but on the periphery in the latter. In *13190* this peculiar pair was not definitely recognizable in most of the plates examined (fig. 19). The plates of *13273* (fig. 18) were the most satisfactory, and the following characterization is based upon them: 2 very long with submedian constrictions, 8 with median, 12 with submedian, and 2 with subterminal. Two of the medianly constricted chromosomes in addition possess terminal con-

strictions ending in fairly large trabants. In some of the medianly or submedianly constricted chromosomes, the constricted part may become drawn so fine that the two portions separate. This provides an explanation for the occasional counting of 25 or 26 chromosomes.

H. parryi Greene. 2n–24.—The plants studied, *13277* and *Keck 1291*, were originally secured west of Alviso and at Salada Beach respectively. The somatic complements are noteworthy for the presence of two very long chromosomes which are medianly constricted and which apparently often fragment at this juncture, so that in many plates 26 chromosomes were counted (fig. 20; the long pair is at the lower left). Ordinarily only a single large trabant is to be observed.

H. pungens T. & G. n–9, 2n–18, with irregularities at meiosis.—Eleven different collections were examined: *12847*, *12907*, *12959*, *13192*, *13260*, *13264*, *13278*, *13279*, *J. J. Walters* at Bard and at Little Shasta River, and *DAJ 689*. The somatic chromosomes are short, thickish, more or less sharply bent or curved, and most of them apparently possess more than one submedian constriction (fig. 21). This character is more pronounced in some of the plants than in others; only a laborious statistical survey of innumerable plates could provide a basis for the morphological classification of the chromosomes. Two terminally long-constricted chromosomes carry large trabants. In two clear cases only 17 chromosomes could be counted. Meiosis has revealed many irregularities: cytomixis is common in the collection from Bard; irregularities in the distribution of the chromosomes to the poles at the first division are common; bivalents are irregularly oriented on the spindle; and often a small blob of chromatin is found in the cytoplasm at the first anaphase or telophase. The figure (fig. 22) is from a plant in which behavior was regular and normal. Rarely two to four univalents may be seen. In one plant, only 8n chromosomes were counted in many of the microsporocytes; this was doubtless due to simple fusion, since one chromosome was greatly swollen. At late metaphase or early anaphase of the first division in *12847*, it was interesting to observe that in most of the cells there were four bivalents with one chiasma and five with two chiasmata; less frequently there were three with two chiasmata and six with but one chiasma.

H. pungens: the "*maritima*" form (=*Centromadia maritima* Greene). n–9, 2n–18, with evidence of fragmentation.—Three series of plants were examined, all coming from the region on both sides of the southern arm of San Francisco Bay: *13259*, *13261*, *13262*. In the somatic complements of *13262*, no evidence of trabants was found (fig. 25), while the plants of the other two groups all showed a single chromosome with one such appendage (figs. 23, 24). In the latter plants, moreover, two or three chromosomes are subterminally shallowly constricted at one end and similarly long-constricted at the other end (fig. 23), separating off a large subglobular piece. This portion may become detached, whereupon it apparently changes its shape to resemble a short chromosome (fig. 24). As there is a total of 21 chromosomes in many plates, some other chromosome has apparently (probably medianly) fragmented (fig. 24).

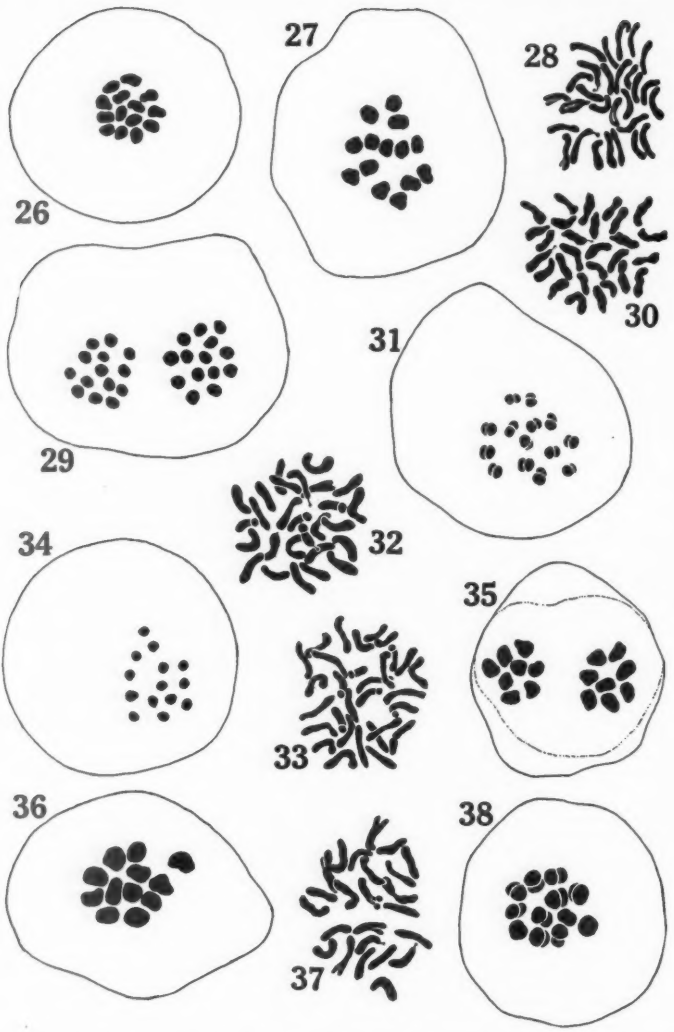
Section EUHEMIZONIA Gray

The section contains two species which differ greatly in their chromosome numbers, but the number has not been found to vary within the individual species.

H. congesta DC.—The species is divided into six subspecies, of which all except ssp. *tracyi* Babcock & Hall have been examined. As indicated in a preliminary paragraph, no plants have been found in any subspecies of *H. congesta* with 12n chromosomes, as reported by MANN (1). All the subspecies have consistently shown 14n chromosomes in every plant examined.

H. congesta ssp. *calyculata* Babcock & Hall. n–14, 2n–28.—Several plants of *12945*, found on the Orr's Spring Road, Mendocino County, provided the material. The somatic chromosomes are the morphological duplicates of those of ssp. *lutescens*. A few have submedian or subterminal constrictions. The two trabants are very small and on long threads. The meiotic chromosomes are intermediate in size between those of ssp. *lutescens* or *clevelandi* and *luzulaefolia*, and of all the subspecies are placed closest together at the first metaphase (fig. 26).

H. congesta ssp. *clevelandi* (Greene) Babcock & Hall. n–14.—Buds of *12926* were examined, from the Eel River east of Laytonville. The meiotic chromosomes are well isolated and easily countable (fig. 27); they are of a size with those of ssp. *lutescens*.



FIGS. 26–38.—Figs. 26–35, *Hemizonia* section Euhemizonia. *H. congesta* ssp. *calyculata:* fig. 26, MI (n-14). *H. congesta* ssp. *clevelandi:* fig. 27, MI (n-14). *H. congesta* ssp. *lutescens:* fig. 28, SM (2n-28); fig. 29, MII (n-14). *H. congesta* ssp. *luzulaefolia:* fig. 30, SM (2n-28); fig. 31, MI (2n-14). *H. congesta* ssp. *typica:* figs. 32, 33 from *13079*, showing respectively fusion (2n-27) and fragmentation (2n-30); fig. 34, MI (n-14). *H. wheeleri:* fig. 35, MII (n-8). Figs. 36–38, *Hemizonia* section "Fruticosi." *H. clementina:* fig. 36, MI (n-12). *H. greeneana:* fig. 37, SM (2n-24); fig. 38, MI (n-12). ×2200.

H. congesta ssp. *lutescens* (Greene) Babcock & Hall. n–14, 2n–28. —The plants which provided the material, *13104*, came from Bodega Bay. As with ssp. *typica*, it is impossible to determine much of the intimate morphology of the somatic chromosomes (fig. 28), although there is considerable evidence of submedian constrictions in most of the chromosomes and the attachment fibers appear to be located at these points. No irregularities in mitosis were noted. Two medium sized trabants occur. There were no clear polar views of the first meiotic metaphase in the available material, consequently a similar aspect of the second metaphase is being presented (fig. 29).

H. congesta ssp. *luzulaefolia* (DC.) Babcock & Hall. n–14, 2n–28. —Four groups of specimens provided abundant material. In most of the somatic complements in all of the plants the chromosomes were too close together and too much intertwined for wholly accurate counts. As the chromosomes themselves are rather small, it was difficult to obtain positive evidence of the occurrence of fragmentation when the total number appeared to exceed 28. The trabants are exceedingly tiny, but could always be located (fig. 30). The meiotic chromosomes are spaced far apart (fig. 31), and are a little larger than those of ssp. *typica*.

H. congesta ssp. *typica* Babcock & Hall. n–14, 2n–28, with apparent fusion and fragmentation.—The three plants studied were: *13076* from the Gallinas Valley, Marin County, *13079* northwest of Petaluma, and *13263* on the Bodega Road, Sonoma County. It is very difficult to judge the true number of chromosomes in most of the somatic complements, principally because of overlapping and intertwisting. In all the root tips examined, the range in number was from 24 to 30. In the clearest plates, 28 were indubitably counted; when the number drops to not more than 27 (fig. 32), one suspects the occurrence of fusion, and when it exceeds 28 (fig. 33), the presence of shorter elements indicates that fragmentation took place. Although at anaphase practically all the attachment constrictions are submedian, there is little evidence of constrictions at metaphase. Two large trabants are present. The very small meiotic chromosomes are always widely separated at the first metaphase (fig. 34), so that exact counts are readily made; no variations in the number have been noted.

H. wheeleri Gray. n–8.—The buds were secured from garden plants late in the season; it was impossible to obtain satisfactory root tips. The plants studied, *13820*, were originally collected at Mineral King. The meiotic chromosomes are large but more easily countable at the second metaphase (fig. 35).

Section "FRUTICOSI"

The section consists of six perennial species, mostly insular, of which those examined possess the same chromosome numbers and seem to be very close together cytologically.

H. clementina Brandegee. n–12, 2n–24.—The two sets of plants available, *Heusi 110* and *111*, were originally obtained on Santa Catalina Island. Estimations of the somatic number were difficult to make; each of the 24 chromosomes has a deep and rarely long submedian constriction, at which point the shorter arm is bent around and often twisted, and as a rule the elements are badly crowded together. The meiotic chromosomes are comparatively large but of the same size throughout (fig. 36).

H. greeneana Rose. n–12, 2n–24.—The plants were grown from seed secured by J. T. HOWELL on Guadalupe Island, Mexico (*S315*). The somatic chromosomes are too short and thick for the facile identifications of constrictions (fig. 37), but at least two pairs plainly show submedian ones. The two chromosomes bearing the terminal trabants are easily located. Meiosis shows some irregularity; the bivalents separate early in the first metaphase (fig. 38).

Section HARTMANNIA Gray, *pars*

The section is the largest in the genus, but it is problematical how many species it contains because of the extreme difficulty of establishing demarcatory lines between certain of the species, notably *H. virgata* and *H. heermanni*, on both taxonomical and cytological grounds. The section is unique for the wide variations in chromosome numbers to be found amongst its various species.

H. angustifolia DC. n–10, 2n–20.—About 22 distinct groups of plants were examined; all proved to be cytologically homogeneous. There is considerable variation in the bulk and size of the somatic chromosomes in the different plants, as might be expected. They may be either short and plump or long and narrow, more or less

curved, with at least two pairs definitely shorter than the remainder, but with no certain evidence of constrictions or trabants (figs. 39, 40). The meiotic chromosomes are large, two of them seeming to be a trifle less bulky than the others (fig. 41).

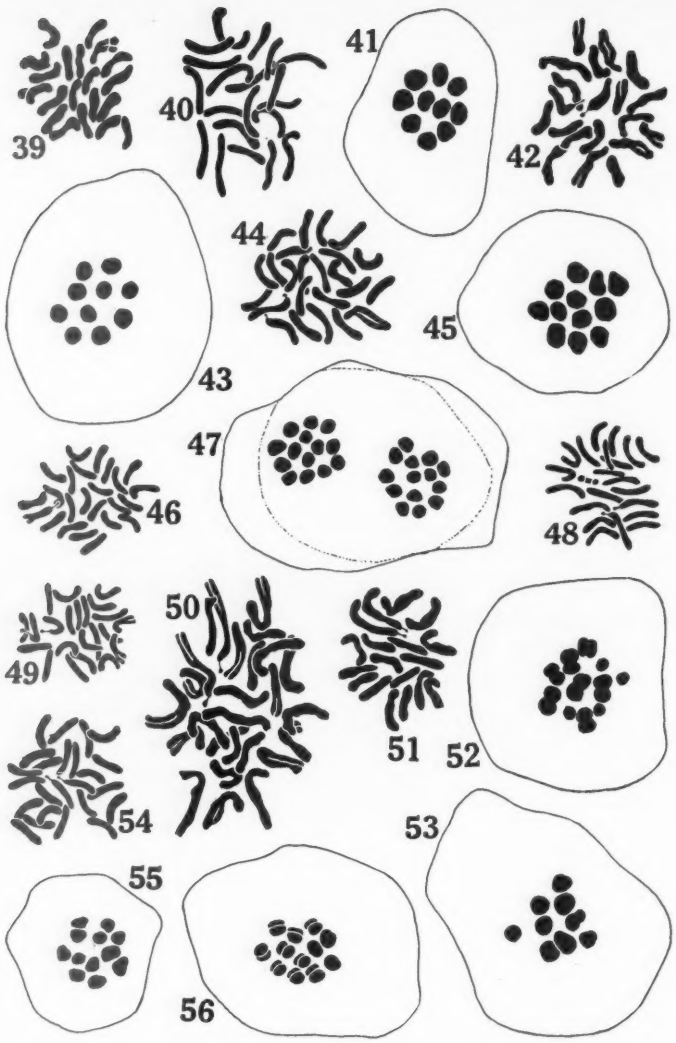
H. angustifolia: the "*macrocephala*" form (=*H. macrocephala* Nutt.). n–10, 2n–20.—This subspecies occurs along the coast in northwestern San Luis Obispo County, the three groups of plants examined coming from San Simeon (*13150*) on the north, through Cambria (*13153*), southerly to Morro (*13140*). Both the somatic (fig. 42) and the meiotic (fig. 43) chromosomes are similar to those of the typical species, but in the somatic set *macrocephala* always possesses a pair with terminal constrictions ending in small trabants. Size differences appear to be somewhat more pronounced in the subspecies. Meiosis is perfectly regular.

H. fasciculata (DC.) T. & G.—This species is composed of a number of forms which were provisionally separated by HALL as distinct subspecies. It soon became evident, however, that the subspecies did not all contain the same chromosome numbers. It is clearly apparent that far more detailed studies upon a large number of individuals are necessary before any taxonomic conclusions with a cytological basis are possible.

H. fasciculata ssp. "*ramosissima.*" This is equivalent to the variety *ramosissima* (Benth.) Gray. n–12, 2n–24.—Eight groups of plants were examined and found to be consistent cytologically. The somatic chromosomes are rather long with perfectly smooth contours. In all the plants save one, two elements were abruptly and terminally short-constricted to bear small trabants (fig. 44). The meiotic chromosomes at the first metaphase are regularly organized (fig. 45).

H. fasciculata ssp. "*superfasciculata.*" 2n–24.—Only the root tips of one group of plants, *13019*, from Ramona, were examined in 1931. The somatic chromosomes (fig. 46) are similar to those of ssp. *ramosissima* but smaller, and usually only one chromosome bears a trabant.

H. fasciculata ssp. "*lobbi.*"—This subspecies is the var. *lobbi* (Greene) Gray. Three groups of plants were examined: *12844* from Livermore Valley, *13043* from Cook P.O., San Benito County, and



FIGS. 39–56.—*Hemizonia* section Hartmannia (*pars*). *H. angustifolia:* fig. 39 (chrom-acetic fixation) and fig. 40 (Nawaschin's), SM (2n-20); fig. 41, MI (n-10). *H. angustifolia* ssp. "*macrocephala*": fig. 42, SM (2n-20); fig. 43, MI (n-10). *H. fasciculata* ssp. "*ramosissima*": fig. 44, SM (2n-24); fig. 45, MI (n-12). *H. fasciculata* ssp. "*superfasciculata*": fig. 46, SM (2n-24). *H. fasciculata* ssp. "*lobbi*": fig. 47, MII (n-14); fig. 48, SM (2n-22). *H. fasciculata* ssp. "*hispidula*": fig. 49, SM (2n-22). *H. floribunda:* fig. 50, SM (2n-26). *H. kelloggii:* fig. 51, SM (2n-18); fig. 52, MI showing 7 bivalents and 4 univalents. *H.* "*pallida*": fig. 53, MI (n-9). *H. paniculata:* fig. 54, SM (2n-24); figs. 55, 56, MI (n-12) (fig. 55 is from *13041* and fig. 56 from *13134*). ×2200.

DAJ 691 from 6 miles north of San Benito. In the last collection meiosis was found to be extremely irregular, and as it was thought to have been influenced by the high temperatures prevailing at the time of collecting and that fixation might have been rendered inadequate by the excessive stickiness of the buds, this lot of material was discarded. In *12844*, buds only were available in 1931 and showed the meiotic chromosomes to number 14; the second metaphase plates were more suitable for countings (fig. 47). In *13043*, the root tips all possessed 22 diploid chromosomes (fig. 48), although on two plates not more than 20 could be counted. In most of the plates two pairs are submedianly constricted and two small trabants are present. The subspecies appears to be a good one, yet there seems no way at present of explaining the two widely different sets of chromosome numbers, 11n and 14n.

H. fasciculata ssp. "*hispidula*." 2n–22.—In this group only root tip counts are being reported, because of the unsuitability of nearly all of the bud material preserved. Three groups of plants were examined: *13205* (4 miles north of Bradley), *13042* (Atascadero Hills), and *13206* (2.2 miles west of Atascadero). In the first two collections, the 22 chromosomes were identical in size, while those in *13206* were much larger, being nearly as large as those in the 24-chromosome sets in other subspecies. In all these 22-chromosome plants only one morphological peculiarity was noticed, namely, that the two chromosomes with trabants have a submedian constriction, at which point the shorter arm is bent around sharply and is terminally long-constricted into the medium sized trabants (fig. 49). In *13206*, two chromosomes possess shallow constrictions but no trabants. It would seem that the behavior of the trabants is deserving of particular attention in future studies on *H. fasciculata*.

H. floribunda Gray. n–13, 2n–26.—The only plants available, *13010*, were originally secured at Potrero, San Diego County. Most if not all of the somatic chromosomes (fig. 50) appear to have submedian constrictions; two are terminally long-constricted to carry fairly large trabants. About four pairs are markedly shorter than the other chromosomes. A considerable number of buds were preserved but all save one had too young flowers; in the one exception the fixation was unsatisfactory. In the few first metaphase plates in

which an approximate estimation was possible, 13n chromosomes were to be seen.

H. kelloggii Greene. n–9, 2n–18.—The one group of plants examined, *13025*, was secured at Aguanga Hills, Riverside County. The root tips were not in the best condition but the accuracy of the count cited is unquestionable (fig. 51). In all of the clear first metaphase figures, the chromosomes were disposed as seven bivalents and four univalents (fig. 52). At the first anaphase, two of the univalents appear to go to each pole undivided.

H. "pallida." n–9.—This undescribed species is closely related to *H. angustifolia* and was for a long time regarded as merely a vernal form of the latter; it is of limited occurrence and distribution. The two species, however, are readily separable on both morphological and cytological grounds. The material was collected from plants growing along the roadside 5 miles north of Grapevine, Kern County (*DAJ 676*). First metaphase stages are exceedingly rare, although diaphases and anaphases are found in abundance. In the one illustrated (fig. 53) there is a distinct gradation in the size of the chromosomes, and the same thing was noticed in the first anaphase chromosomes.

H. paniculata Gray. n–12, 2n–24.—Twenty-four sets of plants from various widely separated localities were investigated; all of these except one agreed among themselves in cytological details. One form, *13134*, from 2.7 miles north of Lompoc, showed very distinct evidence of fragmentation existing as a usual occurrence as practically all the somatic plates contained between 26 and 28 chromosomes. The meiotic chromosomes of this form (fig. 56), however, totaled 12 and showed no evidence of any irregularities.

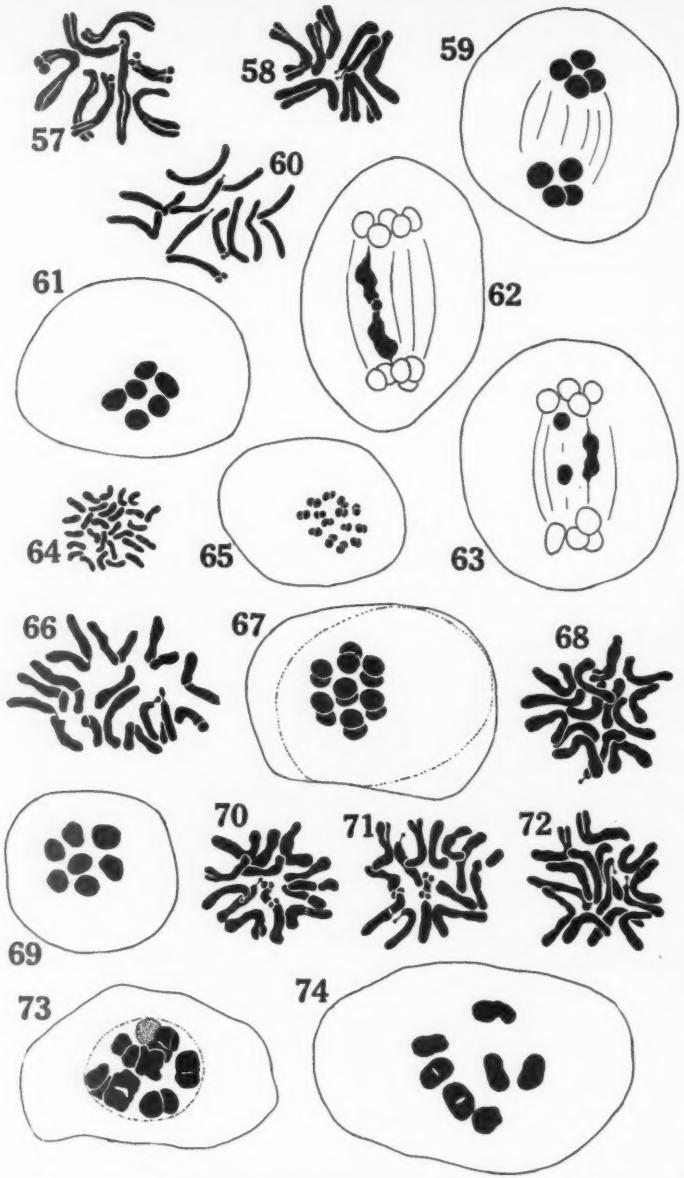
In the majority of the plants the chromosomes displayed no characteristic morphological structure (fig. 54), but in *13284*, from 1.5 miles north of Temecula, four of the chromosomes each showed a deep submedian constriction. This material appeared to be exceptionally well fixed. In *13125*, from Santa Maria, many of the metaphase plates seemed to have one more or one less than the normal number. In all the plants the two terminally constricted chromosomes, which are almost invariably at or near the center of the plate, bearing the small trabants, are easily recognized.

No irregularities of any sort were noticed during meiosis (fig. 55), but in certain more robust plants the chromosomes are twice the bulk of those in less sturdy individuals.

H. paniculata ssp. "*increscens*." 2n–24.—The only recognizable difference between this undescribed subspecies (*13244*, from 4.4 miles north of Salinas) and the species is that the termini of the somatic chromosomes are short-attenuate rather than obtuse. Other morphological details are as described for the species.

H. virgata Gray and *H. heermanni* Greene.—Exactly how to distinguish between these two species is at the moment an unsolved problem. Dr. HALL, upon more than a few occasions, voiced his great perplexity concerning them; he was strongly of the opinion that they belonged together yet could not bring himself definitely to unite them, and was hopeful that the cytological investigation of each might provide a way out of the impasse. Four forms of *virgata* each had 4 haploid and 8 diploid chromosomes, while five of *heermanni* showed 6 haploid and 12 diploid chromosomes; hence it appeared that the two species could be retained as distinct on the basis of that difference in chromosome numbers. This presumption, however, was revealed as fallacious when early in 1933 an indubitable form of *virgata* was discovered by J. CLAUSEN to possess the *heermanni* chromosomal complement. Perhaps the best temporary solution of the problem is to assume that *virgata* and *heermanni* together constitute a single species possessing two distinct cytological forms, plus a third form which has the taxonomical characters of one species but the chromosomal number of the other.

The chromosomes of *H. virgata* may be identified as one very long pair with median constrictions, two pairs with submedian constrictions, plus another pair carrying trabants but which do not appear to be constricted elsewhere than at the terminus (figs. 57, 58, which are from different plants). In *H. heermanni* the somatic chromosomes are narrower, the trabant-bearing pairs are similar to those in *virgata*, and there are likewise two pairs with distinct submedian constrictions, while the remaining three mostly short pairs show no evidence of constrictions (fig. 60). Except for the smaller size of those in *heermanni*, the meiotic chromosomes are similar in the two forms (figs. 59, 61).



FIGS. 57–74.—Figs. 57–65, *Hemizonia* section Hartmannia, continued. *H. virgata:* fig. 57, SM (2n-8), from *13014;* fig. 58, SM (2n-8), from *12885;* fig. 59, late AI (n-4). *H. heermanni:* fig. 60, SM (2n-12) (chrom-acetic fixation); fig. 61, normal MI (n-6); figs. 62, 63, from *12968*, showing irregularities in first division. *Holozonia filipes:* fig. 64, SM (2n-28); fig. 65, MI (n-14). Figs. 66–74, *Lagophylla. L. dichotoma:* fig. 66, SM (2n-14); fig. 67, MI (n-7). *L. glandulosa:* fig. 68, SM (2n-14); fig. 69, MI (n-7). *L. ramosissima:* figs. 70, 71 (from *13093*), showing fragmentation of somatic chromosomes; figs. 73, 74: diaphase and transition from diaphase to MI (n–7); some gemini with 2 chiasmata. ×2200.

In *12968*, a true *heermanni*, there are marked irregularities during meiosis. Many mother cells do not undergo meiosis. Lagging chromosomes, delayed separation of bivalents, and similar phenomena are not uncommon (figs. 62, 63).

Holozonia Greene

The sole species, *H. filipes* (H. & A.) Greene, is a low perennial herb. The only collection available, *13083*, came from Boyes Springs, Sonoma County. Of this, several individuals were examined. The somatic chromosomes, totaling 28, are very small, botuliform, and of even contour (fig. 64). Trabants were not definitely identifiable. The 14 meiotic chromosomes, at the first metaphase, are perhaps the smallest in the tribe (fig. 65).

Lagophylla Nuttall

Included in the genus are three slender annual species, all of which have been investigated.

L. dichotoma Benth. n–7, 2n–14.—In the single set of plants studied, *13158*, from the San Benito Valley, three different individuals were wholly consistent in cytological details. In the somatic set (fig. 66) the most striking morphological feature is the very pronounced median constriction of a single pair, each of whose lengths is longer than that of any others. In addition, three other pairs possess more or less pronounced submedian constrictions; the shorter arm is usually bent around to give the chromosome a J-shape. The pair bearing the large, slightly elongated trabants are distinctly the shortest in the set. The meiotic chromosomes (fig. 67), which are large in consonance with the large size of the somatic set, are regularly constricted and display no recognizable morphological differences.

L. glandulosa Gray. n–7, 2n–14.—With this species, as with the preceding one, only one collection was available, *Keck 1348*, from 2 miles southwest of Yosemite Junction. Five plants were examined and all were essentially identical cytologically. The somatic chromosomes exhibited no morphological peculiarities (fig. 68), in direct contrast to those possessed by *L. dichotoma*. Two fairly large trabants are regularly present, although one is obscured in the figure.

The meiotic chromosomes are large and indistinguishable from one another (fig. 69). The microsporocytes are comparatively small.

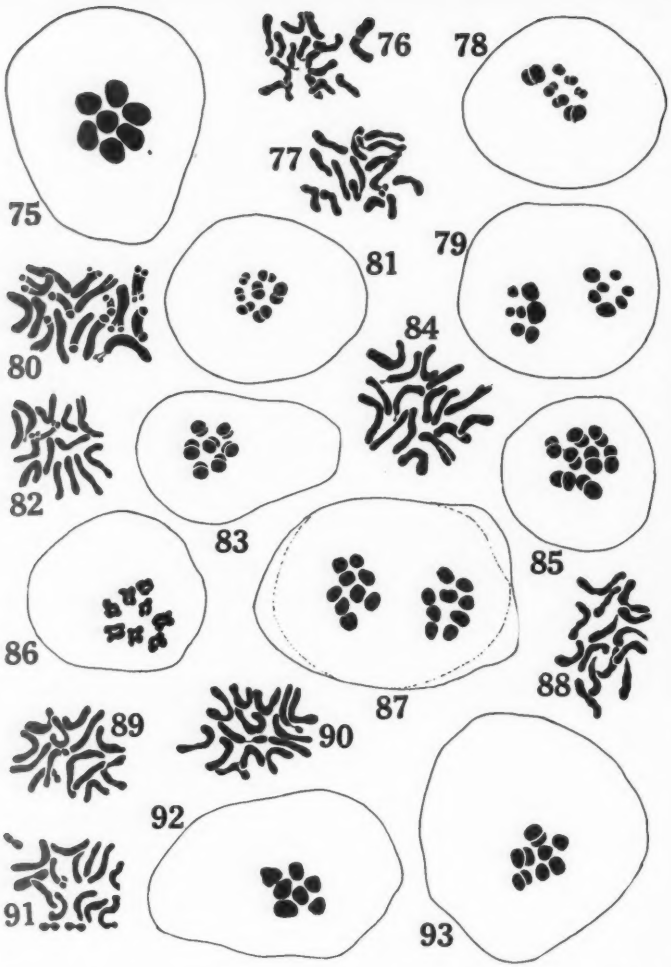
L. ramosissima Nutt. n–7, 2n–14, with fragmentation.—The following collections have been investigated: in 1931, *12910*, *12980;* in 1932, *Keck 1323*, *13093*, and *DAJ 690*. These covered a wide range of territory, extending from Grants Pass, Oregon, to Tehachapi Pass in southern California. Morphologically the somatic chromosomes are decidedly irregular in outline, being unevenly thickened. In the majority of the plates constrictions are difficult to identify (fig. 72), but in some plants of *Keck 1323* a rather high proportion of the chromosomes possess deep median to subterminal constrictions; at such places the chromosomes are often curved or bent abruptly. This species is noteworthy for the all but universal occurrence of fragmentation in its somatic chromosomes; there was hardly a plant which did not show more or less evidence of this phenomenon. It was in consequence possible to follow out all the details of the process, from the beginning of the constriction within the pellicle, with the two segments either sharply detached or connected together by a fine thread, until the final rupture of the pellicle and complete separation (figs. 70, 71). The length of the segmented portion is somewhat variable, generally being a trifle over a third of the whole length of the original chromosome. The total number of chromosomes in any one plate which may fragment is not known, but probably does not exceed four. Fusion (or perhaps merely mechanical cohesion) apparently occurs also, because of the counting of not more than twelve chromosomes on at least four occasions. In contrast to the other species of *Lagophylla*, the trabants of *L. ramosissima* are very tiny and attached at the ends of long, slender threads. The haploid chromosome number does not vary, and there is little or no morphological individuality, but it is to be noted that some of the bivalents have two chiasmata (for example, figs. 73, 74).

Layia H. & A.

The genus consists of approximately 15 species, of which eight have been studied. The species are all annuals, blooming in early spring. There is too close a resemblance between the species for them to be arranged in sections.

Layia jonesii Gray. n–7, 2n–14, with some evidence of fragmentation.—This species, *13144*, is found along the coast near Morro. Four individuals were examined. In most of the root tips, satisfactory plates were exceedingly rare, and even where the chromosomes were clearly separated, they were usually in a condition unsuited for morphological study. The chromosomes in one plant were distinctly smaller and more rounded up than in the other three. It appears that there is at least one pair with nearly median, and two pairs with subterminal, constrictions. The trabants are sometimes conspicuous. In practically every plate in one of the plants, a single chromosome bears a long constriction reminiscent of the type common in *Crepis*. In a few of the plates of this same plant, there was some evidence of fragmentation (or at least of the presence of more than 14 chromosomes), but as it was not observed in the other plants, it is difficult to say whether this phenomenon is as prevalent in this new species as it is, for example, in *L. platyglossa*. The meiotic chromosomes at the first metaphase are large, but apart from this nothing peculiar has been observed (fig. 75).

L. calliglossa (H. & A.) Gray. n–7, 2n–14, with occasional fragmentation.—Two forms, *13081* from El Verano, Sonoma County, and *13084* from Napa County, were studied intensively. Although on the whole the two forms possess the same number of chromosomes, there is a recognizable cytological difference between them in so far as the somatic chromosomes are concerned. In the somatic plates of *13081* (fig. 76) are to be found two large chromosomes lying on the periphery of the plate and with sharp median constrictions, two which are probably medianly constricted, four submedianly, and two short ones terminally, the last carrying tiny trabants. The set from *13084* (fig. 77) likewise shows two large chromosomes, but these are subterminally long-constricted; there are also two with median and four with submedian constrictions. Two chromosomes are much shorter than the others, but there are no trabants similar to those in *13081*. The differences between the somatic chromosomes in the two groups of plants therefore reside in the place and nature of the constrictions in the two large chromosomes and in the presence of inconspicuous trabants in one group. Ignoring these characteristics, the somatic chromosomes may be grouped into the following



FIGS. 75–93.—*Layia*. *L. jonesii*: fig. 75, MI (n-7). *L. calliglossa:* fig. 76, SM (2n 14); fig. 77, SM (2n-14). Fig. 76 is from *13081*, fig. 77 from *13084*, fig. 78, MI (n-7) from *13084*, and fig. 79, MII (n-7) from *13081*. *L. carnosa:* fig. 80, SM (2n-16). *L. chrysanthemoides:* fig. 81, MI (n-7). *L. elegans:* fig. 82, SM (2n-14); fig. 83, MI (N-7). *L. gaillardioides:* fig. 84, SM (2n-16); fig. 85, MI (n-8). *L. hieracioides:* fig. 86, MI (n-8). *L. nutans:* fig. 87, MII (n-9). *L. platyglossa:* fig. 88, SM (2n-14) from *13053*. Figs. 89–91 are from *13065*, figs. 89 and 90 are without fragmentation, while fig. 91 shows 2n-13 plus 3 pieces; fig. 92 (*13110*) and fig. 93 (*13152*), MI (n-7). ×2200.

pairs: two large, two fairly large, two small, and one very small. In one plant of *13081* there were a number of clear-cut cases in which 15 and 16 chromosomes were counted. The supernumerary elements were plainly fragments, hence it seems probable that one or both of the large medianly constricted chromosomes may at times become disjoined. In still another plant, 17 chromosomes were counted six times, and 18 twice, which might indicate that chromosomes other than the two large ones may also divide transversely.

In the two groups of plants, however, the behavior at meiosis is identical and reflects to a remarkable extent the differences at mitosis (figs. 78, 79). These are best revealed at the second metaphase; the sizes given for the somatic set are exactly duplicated for the gametic chromosomes (fig. 79, especially in the group at the left).

L. carnosa T. & G. 2n–16.—The plants were grown from seeds taken from vouchers collected by the writer in 1931 on sand dunes between Samoa and Rolph. The somatic chromosomes (fig. 80) are much alike and the longitudinal split appears in late prophase. The chromosomes are considerably curved, but many appear to have approximately median constrictions. In most of the plates it was impossible to locate the trabants; in the complement illustrated, a single chromosome bore two trabants.

L. chrysanthemoides (DC.) Gray. n–7.—Buds were obtained from plants growing along the Skyline Boulevard at the junction with the side road leading to the Broadway section of Burlingame. The meiotic chromosomes (fig. 81) are sharply differentiated by their sizes: there are two large, three medium, and two small bivalent chromosomes.

L. elegans (Nutt.) T. & G. n–7, 2n–14.—The sole set of plants studied, *13052*, was found near Etiwanda. There are some slight differences between the somatic chromosomes (fig. 82). The two trabant-bearing chromosomes are the bulkiest, but in most of the plates one cannot identify these two with entire certainty because the trabants are so tiny as to be unrecognizable in the rather dense cytoplasm surrounding the chromosomes. One pair is certainly constricted at the center while three other pairs are submedianly constricted. In the microsporocytes the chromosomes are unusually

clear at every stage; there are no differences distinguishing them from one another (fig. 83).

L. gaillardioides H. & A. n–8, 2n–16.—Two groups of plants were examined: *13109* from north of Tomales, and *DAJ 685* from along the road leading to La Honda from the Skyline Boulevard. There is little to distinguish either the somatic or the meiotic chromosomes from one another (figs. 84, 85). Constrictions were shown only vaguely in the somatic chromosomes and those seen appeared to be submedian. The tiny trabants are always plainly visible. Meiosis in *DAJ 685* is peculiar in that at the first metaphase and first anaphase a bivalent with two chiasmata was observed in all suitable cells.

L. hieracioides (DC.) H. & A. n–8.—The material was collected on a dry hill-side above Searsville Lake on the Stanford campus, at the same locality as was *Madia exigua*. The chromosomes at the metaphase of the first division appear to possess two chiasmata; they have never been seen in the rounded-up condition (fig. 86).

L. nutans (Greene) Jepson. n–9, 2n–18.—The only collection available, *13087*, was originally found on the Napa Range. The root tips were in such poor condition that exact computations of the chromosome number could be made in only a few cases. Nothing could be made of the structure of the somatic chromosomes, although these seem to be similar to one another structurally. The plates at metaphase of the first meiotic mitosis were unsatisfactory, but those at the second metaphase allowed no doubt concerning the gametic number. The meiotic chromosomes are all of the same size and configuration (fig. 87).

L. platyglossa (F. & M.) Gray. n–7, 2n–14, with fragmentation.—The following groups of plants were investigated: *13053* from the San Bernardino plains, *13065* from Uvas, Santa Clara County, *13110* from a point a mile south of Tomales, and *13120* on the 17-Mile Drive at Point Joe.

The behavior of the somatic chromosomes is so bewildering as to render the formulation of a comprehensive, logical diagnosis very difficult. As a matter of fact, it would appear that a separate description of each set would be necessitated, and that this could be prolonged more or less indefinitely. Consequently only four metaphase plates are being presented: fig. 88 is from *13053*, while figs. 89–91 are from the same root tip of a plant from *13065*. In the first

plate the absence of trabants will be noted. In fig. 89 there are two chromosomes with median, six with submedian, and two with abrupt terminal constrictions, while the other four elements are more or less straight. In fig. 91 most of the chromosomes are straight or slightly curved, lacking distinct constrictions. Two are apparently terminally constricted. One chromosome (in the lower right corner) possesses a subterminal constriction at each end. The three small pieces are probably extra chromosomes rather than fragments, since each is terminally constricted. The chromosomes in fig. 90 are more nearly of the same size amongst themselves than those in the other plates. In other plates there were several counts of 15, and five of 16 chromosomes; and in addition to these supernumerary elements there was other evidence of fragmentation.

Culture *13110* showed scarcely any evidence of fragmentation; *13120* showed some signs, but the differences in size and shape of the chromosomes were most pronounced in this group. Satisfactory plates were plentiful; most of them contained 14 chromosomes, while one gave 13 plus two small pieces and another the same number plus three fragments.

Meiosis is apparently as stable as mitosis is not. In practically every first metaphase plate were observed three large bivalents and four small ones (fig. 92); the larger chromosomes separate precociously (fig. 93), but distribution of all chromosomes is perfectly regular.

Madia Molina

The genus consists of perhaps 18 species, the majority of which have been available for examination. As commonly treated, the genus is divided rather unsatisfactorily into four sections: Eumadia, Anisocarpus, Harpaecarpus, and Madaria.

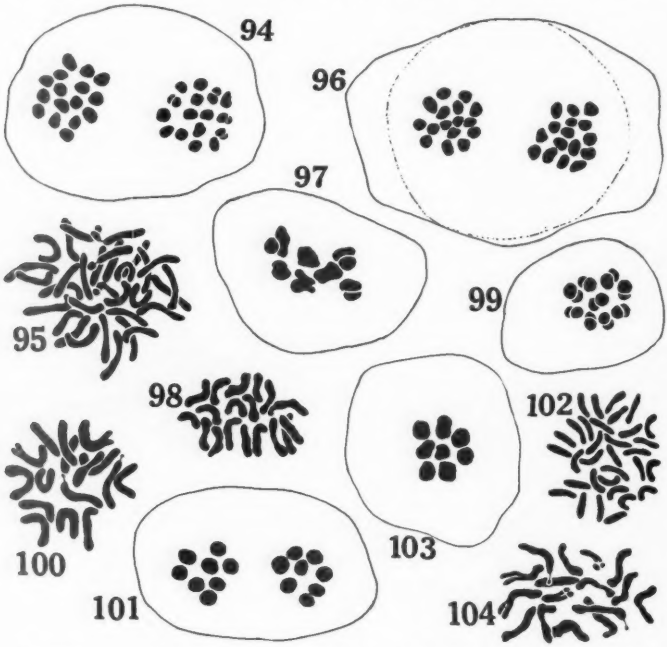
Section "Anisocarpus" (Nutt.)

The section, the largest one, contains seven species formerly included in the genus *Anisocarpus* Nutt. Three species were examined, those not available including *M. bolanderi* Gray, *M. valida* Brandegee, *M. rammii* Greene, and *M. radiata* Kellogg.

M. madioides (Nutt.) Greene. n–7.—The material, *DAJ 680*, was collected in woods on the old Woodside-Kings Mountain grade, San Mateo County. Despite the early separation of the chromo-

somes at the first metaphase, they are situated so far apart that counts are easy (fig. 97).

M. "pumila," an undescribed species. n–9, 2n–18.—The plants which were studied came originally from south of Knoxville. The somatic chromosomes are rather small, crowded, and tend to pair off. The contour is somewhat wavy, which complicates the identi-



FIGS. 94–104.—*Madia. M. dissitiflora:* fig. 94, MII (n-16). *M. sativa* ssp. *"congesta":* fig. 95, SM (2n-32); fig. 96, MII (n-16). *M. madioides:* fig. 97, MI (n-7). *M. "pumila":* fig. 98, SM (2n-18); fig. 99, MI (n-9). *M. yosemitana:* fig. 100, SM (2n-16); fig. 101, MII (n-8). *M. exigua:* fig. 102, SM (2n-32). *M. elegans:* fig. 103. MI (n-8); fig. 104, SM (2n-16). ×2200.

fication of constrictions, which certainly seem to exist. No trabants could be found (fig. 98). The meiotic chromosomes at the first metaphase, as in most of the other species, disjoin early (fig. 99), but are so well separated that counts are thereby facilitated.

M. yosemitana Parry. n–8, 2n–16.—A single group of plants,

13186, from the Hetch-Hetchy Road, provided the material. The somatic chromosomes are bulky, although two pairs are a trifle narrower than the others; some show definite evidence of a median, others of a submedian or subterminal, constriction (fig. 100). Two small trabants occur. The second metaphase configurations (fig. 101) are more satisfactory than those at the first metaphase and were found in abundance.

Section EUMADIA Gray

The section contains five species, of which two have not been available, *M. citriodora* (Gray) Greene and *M. glomerata* Hook.

M. anomala Greene. n–16, 2n–32.—A single collection, *13074*, from Mill Valley, was studied. The root tips and buds were not at all in the best condition, but the accuracy of the counts seems sure.

M. dissitiflora T. & G. n–16, 2n–32.—The following collections have been examined: *13075* from Mill Valley, *13091* from the Napa Range, *13855* from north of Jackson, *DAJ 684* from La Honda, and *DAJ 681* from Grant's Ranch, Mt. Hamilton. The root tips of this species are difficult to manipulate; there are exceedingly few clear plates. The tips were examined before the buds were available and the somatic number was presumed to be 30, owing to the fact that the chromosomes are so prevailingly interlaced and twisted that a positive count was difficult to make. However, after finding many clear first and second metaphase plates (fig. 94), which showed the haploid number to be 16, the root tips were re-examined. There now seems to be little question of the somatic number being other than 32.

M. sativa Molina. 2n–32.—The form studied, *12880*, came from Crow Creek Road, Alameda County. There were but few countable figures in the several root tips examined, but the somatic number is certainly 32.

In addition to the typical *M. sativa*, one subspecies may be recognized. This is the variety *congesta* of TORREY and GRAY. Three collections representative of this form were examined: *13078* from the Gallinas Valley, *13080* from the Sonoma Valley, and 13146 from San Simeon. The cells of the root tips are so small and the chromosomes so numerous that very few clear plates are to be encountered.

In these, 32 chromosomes were seen. Although in most of the plates the chromosomes are without distinguishing features, in root tips of *13080* some chromosomes were seen to have median, others subterminal constrictions. Two rather large trabants are present (fig. 95). The meiotic plates at the second metaphase are clear and show the haploid number to be 16 (fig. 96); the chromosomes at the first metaphase are too much crowded together and disjoin so early that it is difficult to estimate the number accurately.

Section HARPAECARPUS Gray

The section includes only the following species.

M. exigua (Sm.) Greene. 2n–32.—The material was collected from plants growing on a hill-side above Searsville Lake on the Portola-La Honda Road, and from others originally from Muir Gorge (the first collection by the writer, the other by HALL). The somatic chromosomes are small and essentially similar in structure throughout (fig. 102). Trabants were not definitely identified.

Section MADARIA Gray

Included in the section are six species of such close affinity that probably all could be included within the Linnaean species *Madia elegans* Don. The material available for cytological examination would be classified clearly as of that species.

M. elegans Don. n–8, 2n–16.—Plants from six localities were examined: *12867* from Grass Valley, *13020* from Cuyamaca Lake, *13163* from Ackerson Meadow, *13195* from the Indians, Santa Lucia Mts., *13196* from Santa Lucia Peak, and *Keck 1279* from Crystal Springs Lake. A considerable range of territory is represented, although *13195* and *13196* were close together but differed in that the latter is a dwarf form. All six forms were identical in all their cytological aspects, with the possible exception of inconsequential minor morphological variations. The somatic chromosomes are comparatively small, irregularly thickened and contorted, and the presence of constrictions is thus rendered problematical (fig. 104). The trabants are on long, slender threads; this characteristic is more pronounced in *13163* than in the other forms. In decided contrast to the other species in *Madia*, the first metaphase meiotic chromosomes do not separate until late in the stage, but are rounded up and clearly separated (fig. 103).

Summary

In the following list are tabulated the results of the study.

Species	Examined		n	2n	Remarks
	Collections	Plants			
Achyrachaena mollis	3	∞	8	16	No trabants (?)
Hemizonella minima	1	1	ca. 13	ca. 26	
Hemizonia					
Sect. Blepharizonia					
H. plumosa	1	1	14		
Sect. Calycadenia					
H. bicolor	4	8	6	12	
H. ciliosa	4	6	6	12	
H. mollis	4	5	7	14	No trabants (?)
H. pauciflora	1	1	5	10	
H. truncata	4	5	7	14	Meiotic irregularities
ssp. "scabrella"	3	9	7	14	
H. villosa	1	1	7	14	
Sect. Centromadia					
H. congdoni	4	4	.....	24	
H. parryi	2	2	.....	24	One trabant
H. pungens	11	14	9	18	Meiotic irregularities
ssp. "maritima"	3	3	9	18	Fragmentation
Sect. Euhemizonia					
H. congesta ssp.:					
calyculata	1	4	14	28	
clevelandi	1	1	14	.....	
lutescens	1	2	14	28	
luzulaefolia	4	7	14	28	
typica	3	3	14	28	Fusion, fragmentation
H. wheeleri	1	1	8	.....	
Sect. "Fruticosi"					
H. clementina	2	2	12	24	
H. greeneana	1	6	12	24	
Sect. Hartmannia (pars)					
H. angustifolia	22	∞	10	20	No trabants (?)
ssp. "macrocephala"	3	3	10	20	
H. fasciculata ssp.:					
"ramosissima"	8	8	12	24	
"superfasciculata"	1	1	.....	24	One trabant
"lobbi" (Form 1)	2	2	14	.....	
"lobbi" (Form 2)	1	1	.....	22	
"hispidula"	3	3	.....	22	Trabants sometimes lacking
H. floribunda	1	2	13	26	
H. kelloggii	1	1	9	18	Meiotic irregularities
H. "pallida"	1	∞	9	.....	
H. paniculata	24	29	12	24	Fragmentation
ssp. "increscens"	1	1	.....	24	
H. virgata	4	5	4	8	
H. heermanni	5	5	6	12	Meiotic irregularities
Holozonia filipes	1	4	14	28	No trabants (?)

Species	Examined		n	2n	Remarks
	Collections	Plants			
Lagophylla					
L. dichotoma	1	3	7	14	
L. glandulosa	1	5	7	14	
L. ramosissima	5	8	7	14	Fragmentation
Layia					
L. calliglossa	2	7	7	14	Fragmentation; size differences
L. carnosa	1	5	.....	16	
L. chrysanthemoides	1	8	7	.....	Size differences
L. elegans	1	2	7	14	
L. gaillardioides	2	3	8	16	
L. hieracioides	1	8	8	.....	
L. jonesii	1	4	7	14	Fragmentation
L. nutans	1	2	9	18	
L. platyglossa	4	12	7	14	Fragmentation
Madia					
Sect. Anisocarpus					
M. madioides	1	8	7	.....	
M. "pumila"	1	8	9	18	No trabants
M. yosemitana	1	2	8	16	
Sect. Eumadia					
M. anomala	1	1	16	32	
M. dissitiflora	5	8	16	32	
M. sativa	1	1	.....	32	
ssp. "congesta"	3	3	16	32	
Sect. Harpaecarpus					
M. exigua	2	8	.....	32	No trabants (?)
Sect. Madaria					
M. elegans	6	8	8	16	

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HISTOLOGICAL AND REGENERATIVE STUDIES ON THE FLAX SEEDLING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 446

DONALD M. CROOKS

(WITH FORTY-FOUR FIGURES)

Introduction

Common flax, *Linum usitatissimum* L., is the only member of the Linaceae of economic importance (**6, 11**). The fibers are a source of linen, the seed is a commercial source of linseed oil, and the residue after the oil is pressed from the seeds is an important source of food for livestock. Most of the early studies of the plant were made in Europe (**4, 5, 6, 7, 8, 15, 16, 17, 18**). Critical investigations of all phases of fiber development and of the commercial use of flax have been carried on in Holland, Germany, Russia, and in the United States by the Department of Agriculture.

Histological studies on the seedling were confined almost entirely to Europe during the last quarter of the 19th century. GÉRARD (**5**) and TOGNINI (**15**) reported investigations of the transition from root to stem; JANCZEWSKI (**7**) classified the histogens of the primary root; VAN TIEGHEM (**17**) described the origin of the lateral roots; and WILDE (**18**) described transverse sections of the mature root, stem, and leaf. TSCHIRCH and OESTERLE (**16**) described the fruit, reported large aleurone bodies and no starch in the cotyledons, and gave a brief description of germination. In most of these studies flax was selected merely for morphological comparison as a representative of the Linaceae. Certain phases of vegetative regeneration of shoots were observed by REICHARDT (**12**). His observations were verified by TAMMES (**13**) in Holland, and the histology of the regenerated parts was investigated by BEALS (**3**) in the United States. HERZOG (**6**) deals with the botanical, cultural, and commercial aspects of the plant. Extensive bibliographical lists are given by HERZOG (**6**) and TAMMES (**13**). The literature citations included in this paper are

limited to those directly concerned with some phase of seedling histology. The present paper supplements the studies previously made on the histology, anatomy, and regeneration of flax seedlings, and deals in particular with the ontogeny of tissues and organs.

The Bison variety of flax, which is widely grown in North and South Dakota, was used in this investigation. Plants were grown in the greenhouse at the University of Chicago during the winter of 1931–32 and compared with material grown in gardens during the following summer at Ball State Teachers College, Muncie, Indiana. Material from these sources showed no morphological differences except a slightly longer axis in some of the greenhouse plants.

Various fixatives were used. The most satisfactory results were secured with a modification of Nawaschin's solution which consists of solution A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. water) mixed in equal volumes just before using. A solution made of 5 cc. of formalin, 5 cc. of glacial acetic acid, and 90 cc. of 50 per cent alcohol gave fair results except when it was desired to secure mitotic figures and a minimum shrinkage of the protoplasm. The greatest number of division figures were obtained in roots killed at 11 P.M. and in stem tips and leaves killed at 1 P.M. Chloroform was used for clearing. Final infiltration of paraffin was accomplished very slowly, in two stages: (1) by evaporating the chloroform from the paraffin-chloroform mixture in an evaporating dish placed on top of the oven for a period of about 48 hours; and (2) by transferring to an oven at 55° C. until no taste of chloroform remained.

Serial sections were cut 6–15 μ in thickness and stained in a modification of Flemming's triple stain. All drawings (except figure 3) were made by the use of a microprojection apparatus.

Germination and young seedling

Under greenhouse conditions seeds germinated rapidly at a temperature of 65°–90° F. At a temperature of 65°–70° F. the cotyledons showed above the ground in four or five days, and the seedlings appeared much the same as summer-grown plants. Higher temperatures gave more rapid germination, but after a few days the hypocotyl of the seedlings elongated greatly, as compared with those

grown outside during the summer or in cooler temperatures in the greenhouse.

In the process of germination the primary root emerges from the seed coat by the end of the first or second day and grows downward, thus forming a type of tap root which becomes the axis of the entire root system (**2**). Before the cotyledons show above the surface of the soil the hypocotyl region, in response to elongation in the basal portion, bends to the shape of an inverted U, so that the seed coat and cotyledons are pulled above the ground (**10**). Sometimes the cotyledons are pulled out of the seed coat while yet in the soil. The hypocotyl straightens out in about five days and the remainder of the seed coats is pushed off by the enlarging cotyledons. After a period of general growth of the hypocotyl, the first major zone of elongation is at the ground level. This zone of elongation progresses upward along the hypocotyl to the cotyledons, ceasing at the upper level in about 15–20 days. In five or six days several lateral roots appear on the primary root and are usually formed within 3 cm. of the surface of the soil. Most of the lateral roots originate from the upper 8 cm. of the primary root.

As the cotyledons push from the seed coat, they spread apart and continue to expand for several days before the epicotyl begins to elongate. The cotyledons are obovate and almost sessile, and become true photosynthetic leaves. The epicotyl grows very slowly at first. By 12–15 days it appears as a compact bud of miniature leaves with almost no elongation of internodes. After about 20 days the elongation of internodes causes very rapid growth in height. At maturity the first internode is about 1 mm. long, the second about 15 mm., and all others are usually from 5 to 10 mm. Most of the leaves are simple and sessile, although several plants have one or two double leaves between the fifth and tenth node (fig. 25).

Primary root

The primary root is diarch, with three to five well defined protoxylem elements alternating with two rather large groups of small primary phloem cells (fig. 15). The metaxylem elements are successively larger toward the center. The pericycle, which is differentiated early, is a single layer of cells. The Casparian strips of the

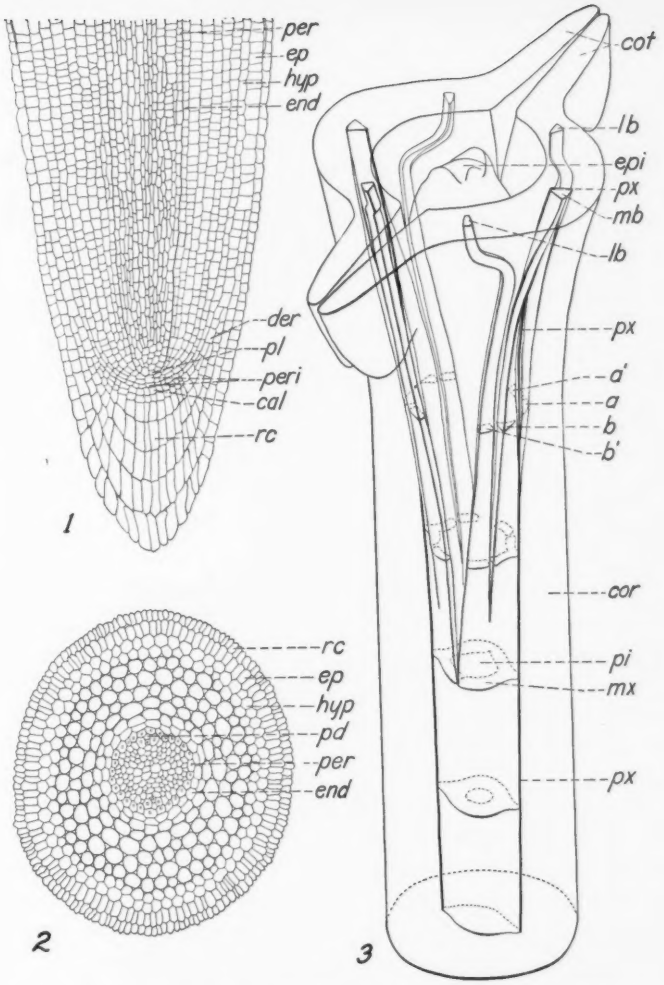
endodermis never become very thick but are deposited on the radial walls before the metaxylem is completely differentiated. The cortical cells are very regular with large intercellular spaces. The outer layer of the cortex forms a definite hypodermis which appears much as the epidermis, except that none of the cells elongate and become root hairs. The cortex persists after considerable secondary thickening, and the first breakdown of cortical cells occurs in the region midway between the endodermis and hypodermis.

Development of the root axis is by clearly defined histogens (fig. 1) which are similar to those described by JANCZEWSKI (7). The plerome and periblem give rise to the stele and cortex respectively; while a layer of cells, which makes up the calyptrogen and dermatogen overlying the periblem, produces the epidermis and the root cap.

The calyptrogen-dermatogen layer of cells overlying the periblem multiplies by periclinal divisions around the tip and forms a root cap of regular, radial rows of cells. In the lateral portion of the same layer the epidermis is formed by anticlinal divisions. Laterally, where the divisions are anticlinal the histogen is strictly a dermatogen, while the layer at the tip is a calyptrogen. The root cap at the tip is very regular because the cell divisions in the calyptrogen are periclinal only. Occasionally some of the cells in the lateral portion of the root cap divide anticlinally and distort the radial rows. This occurs only in lateral portions of the root cap and not at the tip, where the rows of cells are regular.

The cortex is derived from the periblem, which consists of two layers of cells overlying the plerome. The outermost layer of the periblem divides only anticlinally and forms the outer layer of the cortex, the hypodermis. The inner layer divides in all planes and forms the remainder of the cortex, which at maturity is about 6–9 cells in thickness (figs. 1, 2).

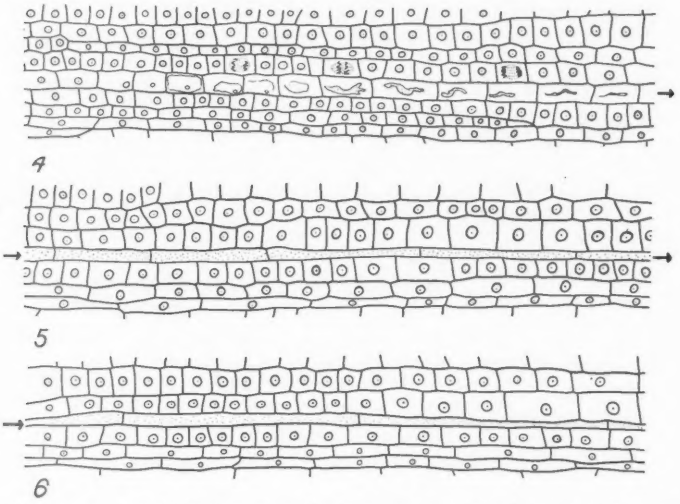
The stele is differentiated from a small group of plerome cells which divide in all planes at the tip, while divisions at a higher level are chiefly in a transverse plane. The outer layer of this group produces the pericycle, which can be distinguished rather early by the relatively dense cytoplasm and larger size of the cells. The first evidence of differentiation of the vascular system appears about 0.4 mm. above the tip of the plerome. Two primary phloem ducts are



FIGS. 1–3.—Fig. 1, median longitudinal section of primary root: *per*, pericycle; *ep*, epidermis; *hyp*, hypodermis; *end*, endodermis; *der*, dermatogen; *pl*, plerome; *peri*, periblem; *cal*, calyptrogen; *rc*, root cap. Fig. 2, transverse section 0.7 mm. from tip of primary root: *pd*, primary phloem duct.

Fig. 3, schematic diagram of vascular system of hypocotyl showing transition from root to cotyledons: *cot*, basal portion of cotyledons; *lb*, lateral bundle of cotyledon; *epi*, epicotyl; *mb*, median double bundle of cotyledon; *px*, protoxylem; *mx*, metaxylem; *pi*, pith; *cor*, cortex; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a'*, *b'*, lateral metaxylem elements which form lateral bundles of cotyledon.

first differentiated opposite each other by the elongation and breakdown of a single row of cells lying next to the pericycle, and alternate with the two protoxylem points which are differentiated later. Figures 4, 5, and 6 show how these ducts are formed and stretched by the elongation of neighboring cells. These ducts collapse after becoming greatly stretched but usually persist until the protoxylem elements are well differentiated. Elongation of the root often causes



FIGS. 4–6.—Longitudinal sections of primary root showing formation of primary phloem duct at 0.6, 1, and 1.5 mm. from tip.

the protoxylem to collapse. The metaxylem elements differentiate centripetally until all the elements of the xylem plate are lignified and no central parenchyma remains. The outermost metaxylem elements have scalariform wall thickenings while those centrally located have pitted wall thickenings.

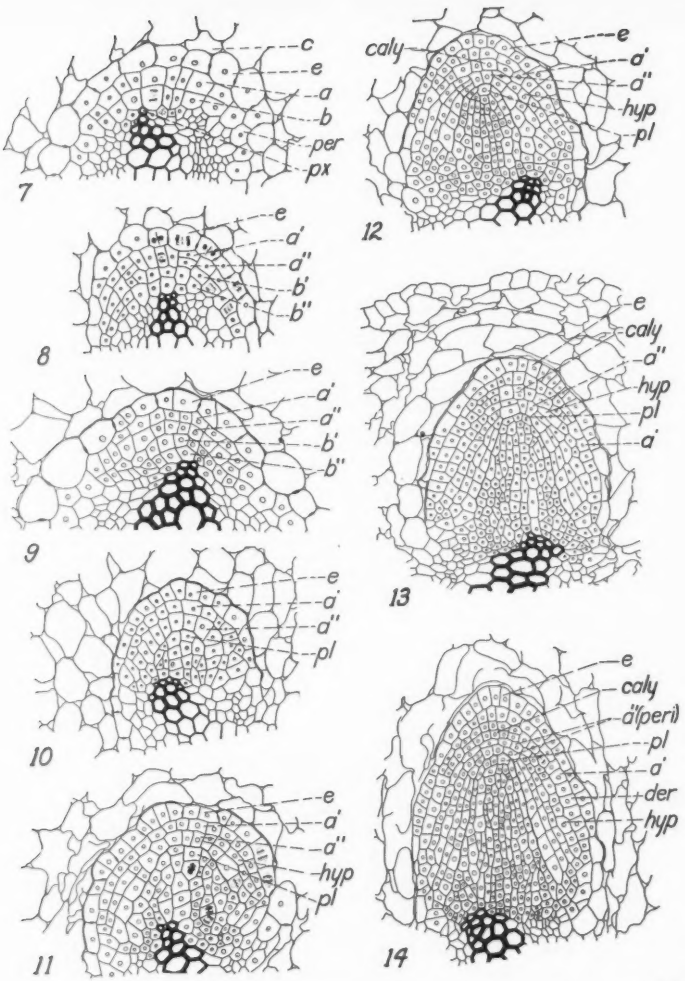
Lateral root histogens

Lateral roots are formed in the ontogeny of the primary axis about the time the thickenings are deposited on the walls of the metaxylem cells. Several lateral roots develop just below the soil level and are usually oriented slightly to one side of the protoxylem point, but

may occur on the same radius directly outside this point (**9**). Development is initiated by a tangential division of several cells of the pericycle next to a protoxylem point (fig. 7). This division produces two distinct layers (*a*, *b*), each of which in turn again divides by another tangential division (figs. 8, 9). The inner layer (*b*) usually divides somewhat in advance of the outer layer (*a*), as is indicated in figure 8. Figure 9 shows the second tangential division completed, forming four layers of cells, the outer two (*a'*, *a''*) being derivatives of (*a*) and the inner two (*b'*, *b''*) being derivatives of (*b*). A few radial divisions which compensate for the increase in length of overlapping layers occur in the two- and four-layered pericycle, but these divisions usually cause no distortion of the tangential rows. The outer (*a'*) of the four layers derived from the pericycle gives rise to the calyptrogen and dermatogen; the next (*a''*) gives rise to the periblem; and the two inner ones (*b'*, *b''*) continue cell activity as a plerome.

By the time four layers of cells have been derived from the pericycle, the endodermis becomes active by radial divisions (**9, 17**). At this stage the endodermis can easily be identified by the Casparian strips which persist on the radial walls after several subsequent radial divisions in the original cells. The endodermis continues to divide in a plane anticlinal to the developing lateral root, and forms a uniseriate layer of cells about the tip. This layer produced from the endodermis never becomes more than one cell in thickness. Cell activity continues until the lateral root has pushed through the cortex of the primary root into the soil and the cell layer derived from the endodermis is sloughed off with the outer layer of the root cap.

The four layers of cells derived from the pericycle establish the first histogen of the new lateral root. The inner two layers (*b'*, *b''*) continue to divide in all planes and form a conical group of cells, the plerome (fig. 10). Several subsequent divisions follow in the plerome before any tangential divisions take place (periclinal to the new lateral root) in the outer two layers (*a'*, *a''*). All subsequent development and differentiation of the plerome is similar to that described for the primary root. The primary xylem of the lateral root is diarch, and the protoxylem elements lie in the same plane as those of the primary root from which the lateral root originates.



FIGS. 7–14.—Transverse sections of young primary roots showing origin of lateral roots: *px*, primary xylem; *per*, pericycle; *e*, endodermis; *pl*, plerome; *hyp*, hypodermis; *caly*, calyptrogen; *peri*, periblem; *der*, dermatogen; *a*, outer layer of first two layers derived from pericycle; *b*, inner layer of first two layers derived from pericycle; *a'*, outer layer derived from *a*; *a''*, inner layer derived from *a*; *b'*, outer layer derived from *b*; *b''*, inner layer derived from *b*. Derivatives of layer *a'* become the calyptrogen and dermatogen; derivatives of layer *a''* become the periblem; derivatives of layer *b* become the plerome.

Concurrent with increase in the size of the plerome, several radial divisions (anticlinal to the new lateral root) occur in the two outer layers from the pericycle (a', a''). Following establishment of the plerome, the innermost (a'') of the two outer layers begins periclinal divisions at a point farthest from the tip (fig. 11). The periclinal divisions in this layer (a'') occur successively toward the tip for a time and then their innermost derivatives undergo periclinal divisions. This condition is shown on the right side of figure 11, where the basal part of layer a'' has divided once and the innermost derivative is again undergoing division, as indicated by a mitotic figure. The number of cell layers is increased by successive periclinal divisions of the newly formed innermost layers (fig. 12). Such periclinal divisions are continued progressively toward the tip of the new root, and finally the one to four remaining cells of layer a'' (fig. 13) are divided once periclinally, and the fundamental plan of the periblem (fig. 14) becomes established on the same plan as described for the primary root. The outer layer of the periblem divides only anticlinally, thereby continuing the uniseriate layer which differentiates into the hypodermis. At the tip of the root the innermost layer of the periblem continues to produce, by subsequent divisions, the remaining six to eight layers of the cortex (fig. 14).

By the time the layer a'' and its derivatives have developed to the extent that about three layers of cells are formed about the base of the conical plerome, the outermost layer (a') is divided at the tip by periclinal divisions (fig. 12). This initiates development of a calyptrogen by forming two layers of cells of which the outer is the root cap while the inner remains meristematic, functioning as a calyptrogen and later dividing to form the second layer of the root cap. By such subsequent periclinal divisions the root cap is produced and the calyptrogen is perpetuated as a single layer of meristematic cells overlying the periblem. The laterally oriented cells of the layer a' continue anticlinal divisions as a dermatogen, which is the same as was described for the primary root.

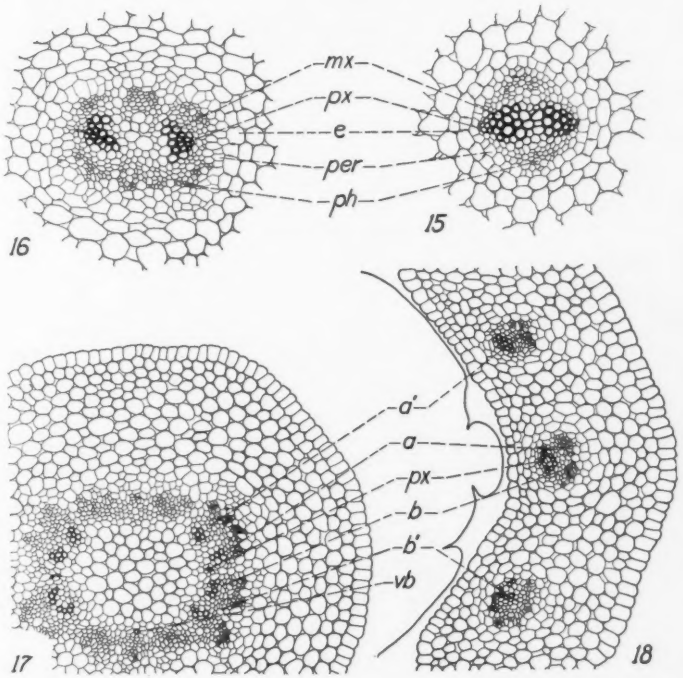
With all the histogens of the lateral root established on the same plan as the primary root, the subsequent growth and differentiation is also as that described for the primary root. There is little accumulation of cell débris or distortion of cortical cells about the tip

of the young lateral root as it grows through the cortex of the primary root. This would seem to indicate that there is some digestion as well as mechanical crushing of the cortical cells as the lateral root develops.

Hypocotyl, cotyledons, and transition

The lower portion of the hypocotyl is rootlike in vascular arrangement. A section made at approximately the ground level shows a primary xylem plate without central pith and two phloem groups alternate with the protoxylem elements (fig. 15). The central portion of the stele of the hypocotyl is parenchymatous at approximately one-fourth of its length above ground. There is a gradual reorientation of the primary vascular tissue at successively higher levels throughout the hypocotyl and basal part of the cotyledons. In the lower one-fourth of the hypocotyl the two phloem groups diverge and form four phloem regions which at a higher level are equally distributed at 90° intervals about a cross-section (fig. 16). Phloem which is associated with the first two leaves of the epicotyl is soon differentiated in the hypocotyl, and anastomoses at the point at which the phloem groups of the lower hypocotyl diverge. In the central region of the hypocotyl the metaxylem elements are differentiated in a tangential position with respect to the protoxylem points. This forms four metaxylem groups, each of which is accompanied by a corresponding phloem strand located outward on the same radius. Somewhat above the middle region of the hypocotyl, the four metaxylem groups with the related phloem groups are each differentiated as two groups, so that eight vascular strands associated with the cotyledons are formed. At successively higher levels throughout the hypocotyl the four metaxylem groups (fig. 3 *a*, *a'*, *b*, *b'*) related to each protoxylem point become oriented more and more definitely lateral to the protoxylem (figs. 3, 17, 18). In the upper region of the hypocotyl this orientation of the eight cotyledonary bundles results in two widely separated groups, each of which becomes a cotyledonary trace. At the point of divergence of a cotyledon the two lateral bundles of metaxylem and accompanying phloem (*a'*, *b'*) of each group of four become widely separated from the two middle bundles (*a*, *b*). Each of these lateral bundles (*a'*, *b'*) is a

bundle of the trace of a lateral vein of the cotyledon. At successively higher levels in the upper hypocotyl and basal part of the cotyledon,



FIGS. 15–18.—Transverse sections of seedling 6 days old showing transition from root to cotyledon. Fig. 15, transection about 1. mm. below ground level. Fig. 16, transection in middle region of hypocotyl showing parenchymatous center of stele and early breakdown of protoxylem. Fig. 17, transection of upper region of hypocotyl showing 8 metaxylem groups with associated phloem (also breakdown of protoxylem). Fig. 18, transection at base of cotyledons and epicotyl showing level where two metaxylem groups anastomose and showing also two phloem groups which retain their identity until a higher level in the cotyledon (two lateral veins of cotyledon also shown): *mx*, metaxylem; *px*, protoxylem; *e*, endodermis; *per*, pericycle; *ph*, phloem; *vb*, vascular bundle of first leaf; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a'*, *b'*, lateral metaxylem bundles which form lateral bundles of cotyledon.

the two metaxylem groups (*a*, *b*) nearest the protoxylem become oriented more and more in an endarch relationship to the proto-

xylem. In the base of the cotyledon the two metaxylem groups are differentiated in an endarch relation to the protoxylem, and at this level the two groups of metaxylem anastomose (fig. 18) and form the median double bundle (fig. 26) of the cotyledon (**14**). The two phloem groups associated with the median bundle retain their identity for 2–3 mm. farther into the cotyledon and then anastomose (fig. 18).

Figure 3 shows the transition in a semidiagrammatic manner. Each strand shown in this diagram may be interpreted as a bundle of associated xylem and phloem. The vascular elements of the epicotyl are differentiated somewhat later than the vascular system related to the cotyledons. Figure 17 shows a zone of phloem which is associated with the first leaves. These bundles are differentiated as endarch, collateral bundles throughout the hypocotyl, where they may anastomose with the lateral arcs of metaxylem and accompanying phloem in the lower hypocotyl, or may end blindly in the parenchyma. The bundles related to the first two leaves are small in the middle and lower hypocotyl, and usually anastomose with the lateral arcs of metaxylem and accompanying phloem; while the other bundles related to the epicotyl end blindly and have vascular connection only through secondary tissue. The transition is actually a root cotyledon transition as there is no reorientation of bundles that have relationship to the epicotyl.

In order to follow the transition in the middle and upper hypocotyl, it is necessary to examine plants before the hypocotyl has greatly elongated. Plants six days old, or when the cotyledons first come above the soil, have well differentiated primary xylem throughout the hypocotyl and cotyledons. As the hypocotyl elongates the protoxylem is crushed and most of it is obliterated by digestion. Later the early metaxylem breaks down and finally nearly all of the primary xylem disappears in the middle and upper hypocotyl. All of the primary xylem elements in the upper region of the hypocotyl have spiral wall thickenings. This is the last region to cease elongation, and the consequent and relatively continuous stretching causes a collapse of the spiral vessels.

By the time plants are 13 days old, most of the primary xylem of the upper region of the hypocotyl is crushed and is partly resorbed (fig. 19). CHAUVEAUD (**4**) has reported breakdown and complete

resorption of primary xylem in *Mercurialis* and *Allium*, much the same as was observed in *Linum*. In *Linum* there are fragments of the primary xylem that remain after 30 days, and may remain through the life of the plant, without being completely resorbed. Many cross-sections through the upper hypocotyl show no protoxylem and very little metaxylem, while at other levels fragments of

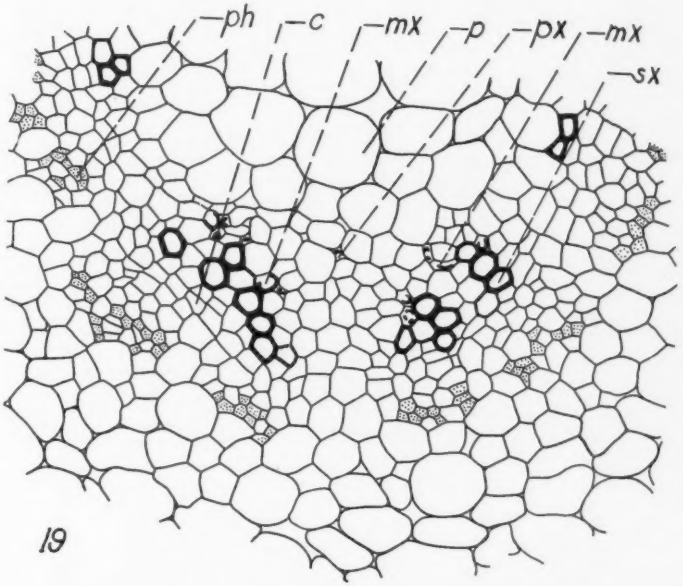


FIG. 19.—Transverse section in upper region of hypocotyl showing early breakdown and resorption of primary xylem: *c*, cambium; *mx*, metaxylem; *px*, protoxylem; *sx*, secondary xylem; *p*, pith; *ph*, phloem.

the elements can be found between the parenchyma cells. The ephemeral nature of the primary xylem probably led to some misinterpretation when GÉRARD (5) and TOGNINI (15) described the transition from root to stem in *Linum*. TOGNINI observed the breakdown of primary xylem but did not distinguish protoxylem and metaxylem in respect to their time and position of differentiation in the upper region of the hypocotyl.

The cotyledons are almost sessile, and in young seedlings they are

somewhat wider than the hypocotyl at their point of divergence. In the young seedlings they are made up of very compact storage cells.

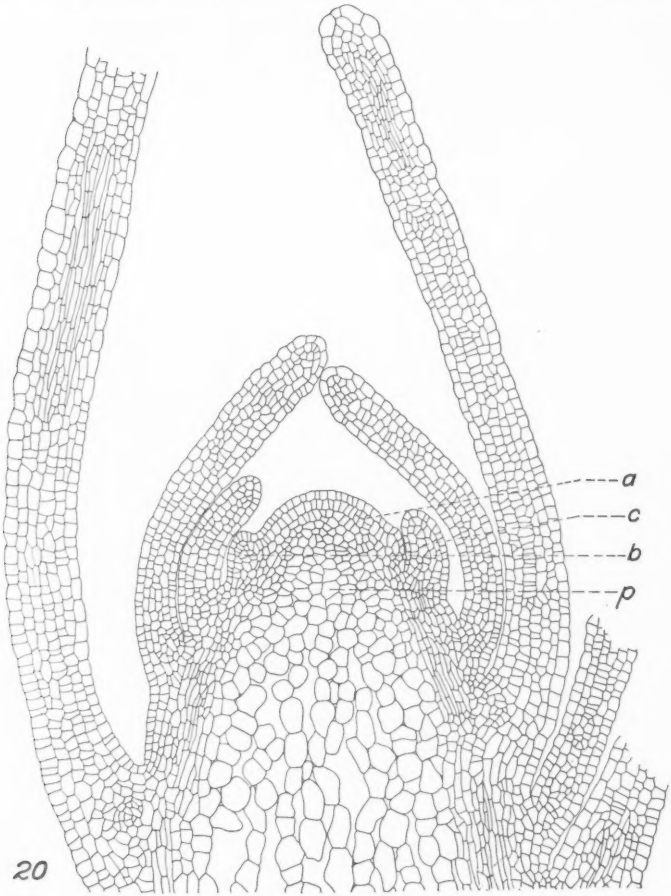


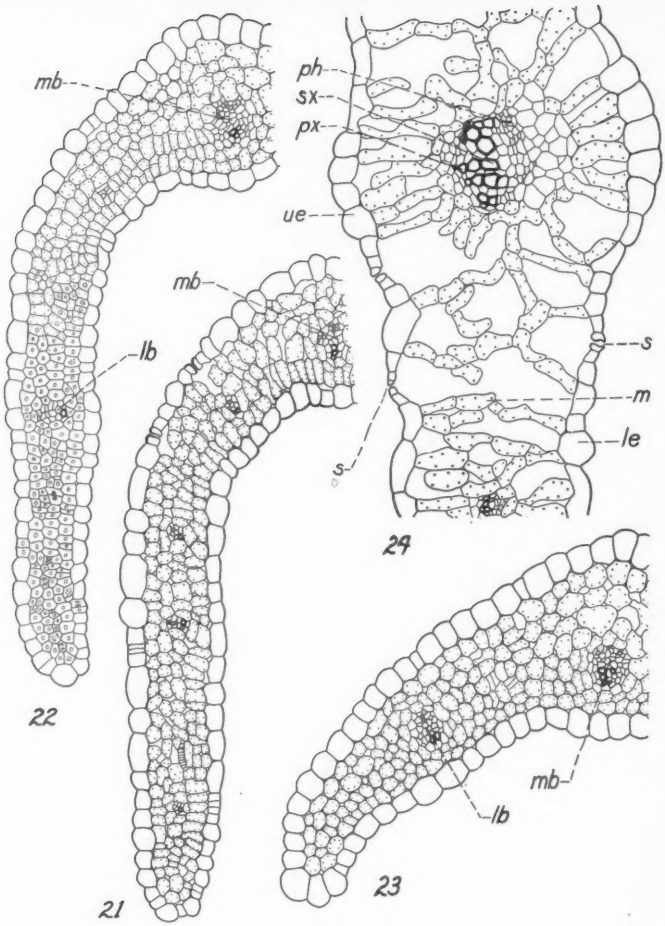
FIG. 20.—Median longitudinal section of growing point showing several stages of ontogeny of leaf: *a*, section of very young primordium; *b*, *c*, older primordia showing early development of provascular strands; *p*, pith.

Two or three of the adaxial layers take the form of a palisade layer, while the lower mesophyll consists of four or five layers of more

rounded, compact cells. Protoxylem elements are differentiated in the median bundle of the cotyledon in seeds placed in conditions favorable for germination for a period of 24 hours, and in some cases may be differentiated in the mature seed. Usually a few scattered phloem cells can be identified in the provascular strands of the young cotyledons before any wall deposits can be noted in the cells of the primary xylem. After the cotyledons are above the ground they continue to thicken and enlarge for a period of about 15 days. The photosynthetic tissue of the mature cotyledon has in general the structure of a mature leaf (fig. 24), except that it is considerably thicker and has smaller air spaces. Both the upper and lower epidermis have a considerable number of stomata with large air spaces underlying them. The closed, netted venation of the cotyledon and leaf is essentially the same (figs. 26, 27). Except in a short region in the median vein, the vascular bundles of the cotyledons are collateral and develop no secondary tissue, while the large leaf bundles develop considerable secondary xylem and phloem. The xylem elements of the cotyledons have spiral or loosely netted wall thickenings. After about 30 days the cotyledons become yellow and die.

Epicotyl and foliage leaves

The young epicotyledonary axis is surrounded by the bases of the cotyledons and develops very slowly for the first eight to ten days. The growing point cuts off leaf primordia which have an irregular phyllotaxy. The leaves at the first three nodes are usually in alternate pairs. Above the fourth node the leaves are arranged spirally, with one leaf at a node in a more or less indefinite phyllotaxy. TOGNINI (**15**) classified the arrangement of the upper leaves in a 2/5 system of phyllotaxy but states that frequently there are exceptions, even in different parts of the same plant. In this investigation no definite phyllotaxy could be established for any one plant, except for short intervals which suggested a 2/5 system. Occasionally three leaves are in a whorl at one of the lower nodes, and often the sixth, seventh, or eighth leaf is bilobed (fig. 25). A bilobed leaf may be broad and cleft almost to the base, or narrow with a very small cleft at the tip. The vascular system of such leaves shows them to have the form of a double leaf or of two leaves nondiverged in varying

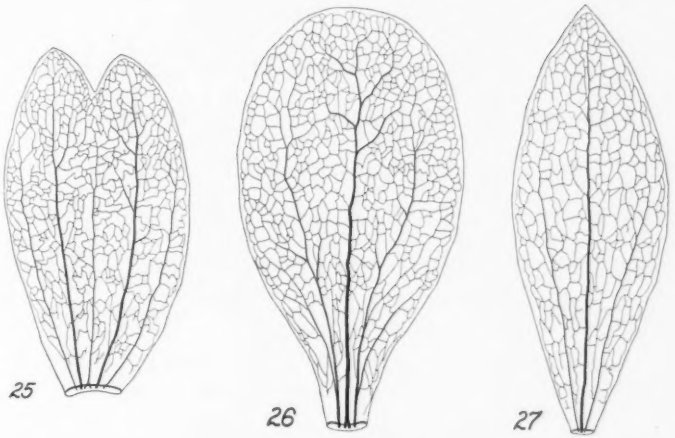


FIGS. 21–24.—Figs. 21–23, transverse sections of first leaf when its total length is 2.5 mm. Fig. 21, transection 0.9 mm. from tip of leaf where cell divisions have ceased. Fig. 22, transection 1.8 mm. from tip of same leaf showing region of meristematic activity in lamina. Fig. 23, transection 0.1 mm. from base of same leaf showing level where cell divisions have ceased: *mb*, median bundle; *lb*, lateral bundle.

Fig. 24, Transverse section through median vein of mature leaf from middle region of stem: *px*, primary xylem; *ph*, phloem; *sx*, secondary xylem; *s*, stoma; *le*, lower epidermis; *ue*, upper epidermis; *m*, mesophyll.

degrees. A double leaf usually has two perfect leaf traces. The lateral bundles of the two traces anastomose and form a small bundle which is median in the double leaf (fig. 25).

A leaf primordium arises from tissues of at least the three outermost cell layers of the growing point and forms a conical mass of cells. It elongates and broadens by general meristematic activity until it becomes a somewhat flattened, finger-like projection. The procambial strands are differentiated early (fig. 20*c*). General meristematic activity increases the length of a flattened primordium to about one-sixth or one-fifth the length of a mature leaf. Continued meristematic activity laterally and toward the adaxial surface results in formation of the lamina (fig. 22). During formation of the lamina other cells of the mesophyll undergo enlargement and further differentiation, and the vascular bundles are more completely differentiated. Meristematic activity first ceases in the tip of the leaf, next in the basal portion, and last in the basal portion of the lamina (figs. 21, 22, 23). After the young leaf has attained one-sixth to one-fifth its mature size all further expansion is by enlargement, stretching, and pulling apart of cells.



FIGS. 25–27.—Diagrams showing venation of cotyledon and foliage leaves: fig. 25, double leaf; fig. 26, cotyledon; fig. 27, typical early foliage leaf.

At maturity the mesophyll is very loose, with large intercellular spaces (fig. 24). There are about four cell layers of somewhat elongated cells which are essentially alike and not differentiated into a definite palisade and spongy parenchyma. In the mesophyll there is a complicated network of veins of various sizes. The median vein and sometimes the two lateral veins develop considerable secondary tissue. The smaller veins have fewer and fewer elements, and either form a closed netted system or may end blindly in the mesophyll. The epidermal cells are irregular in size and have a thin deposit of cutin on the outer walls. The stomata are numerous and occur in about equal numbers on both sides of the leaf. The guard cells are subtended by accessory cells.

The fundamental plan of the primary vascular system of the stem is differentiated early, in the form of procambial strands in the leaf primordia and growing point. The leaf primordia are close together, so that a length of 0.1–0.15 mm. of the growing point includes 10–20 nodes and internodes. The procambial strands are differentiated in the leaf primordia and to a distance of 0.1–0.15 mm. in the meristematic zone of the growing point at essentially the same time (fig. 20 *b*). Even in this short distance the strands extend through 10–20 nodes, and as the internodes elongate the vascular system is elongated with the same fundamental plan and with no differentiation of anastomosing bundles. The mature primary vascular system is then made up of foliar bundles, all of which are collateral. The leaf trace consists of three large bundles which differentiate as separate bundles in the cortex and anastomose at the point at which the trace becomes a part of the vascular ring in the axis. The bundles are largest where they diverge from the stele, and are gradually smaller at lower levels, where they end blindly after extending through 15–20 nodes. The vascular connections within the stele are by secondary tissues and not by anastomosing of bundles. Usually 8–10 foliar bundles are differentiated below the cotyledonary node. Bundles associated with the first pair of leaves anastomose with the lateral arches of the metaxylem of the rootlike lower hypocotyl; but even in this case the bundles are small at the lower level and vascular connection is principally by secondary tissues. Other foliar bundles below the cotyledonary node end

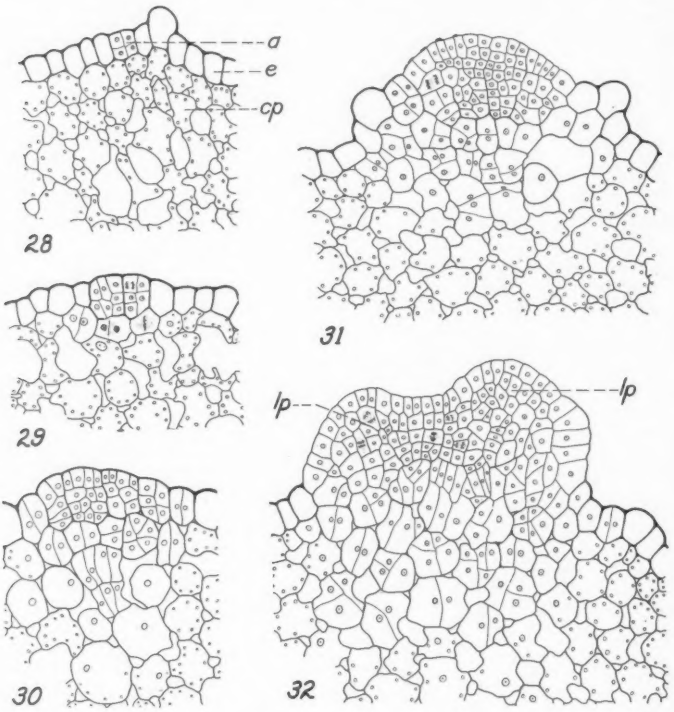
blindly in the parenchyma between other vascular bundles. As the primary tissues mature, fascicular and interfascicular cambium is differentiated. The tissues formed by the activity of fascicular and interfascicular cambium form a continuous vascular cylinder of secondary tissue, which establishes the only vascular connection between the primary vascular elements.

Adventitious buds from hypocotyl

Young plants readily produced shoots when the growing point was injured or removed. When the injury was above the cotyledon an axillary bud became active and produced a shoot. All organized buds were removed by severing seedlings of various ages at different levels below the cotyledons. All plants not more than 10 days old produced 5–20 buds on the remaining lower part of the hypocotyl, even when the top had been removed to a few millimeters above the ground level. When the tops of plants 60 days old were severed below the cotyledons, approximately 60 per cent of the plants died without forming buds; while plants less than 10 days old produced small buds along the remaining hypocotyl within 6–8 days after severing. These buds do not all start development at the same time. A microscopic examination shows that while buds on one part of the hypocotyl are large enough to show small leaves, many other buds are in earlier stages of development. After 8–10 buds begin to show small leaves, one bud usually outgrows all the others. This one bud may be at any level in relation to the others, and is not necessarily the first one to have made its appearance on the hypocotyl. In a few instances more than one bud may continue development. The shoot that develops from such an adventitious bud continues as an unbranched axis until the formation of a flower cluster, which matures at approximately the same time as those of plants of the same age left uncut. At maturity the adventitious shoot has attained about one-half the height of a plant left uncut. Many of the cells at the cut surface of the hypocotyl collapse and die, and little or no wound parenchyma is formed by the underlying cells.

After a plant has been severed, the cortical region of the remaining part of the hypocotyl develops large intercellular spaces which give it an appearance similar to the mesophyll of a leaf (figs. 28, 29).

The bud is initiated by division of an epidermal cell. This first division is quickly followed by a second, which gives a four-celled stage such as is shown by figure 28. Epidermal cells adjoining the derivatives of the first meristematic cell in turn become active, so that



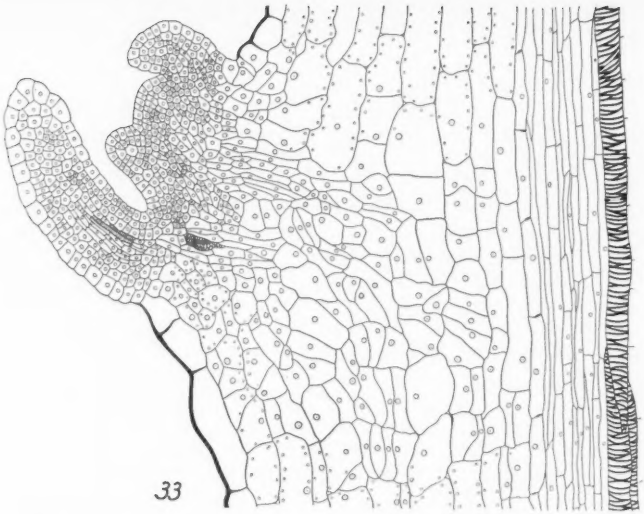
FIGS. 28–32.—Transverse sections of hypocotyl showing development of adventitious bud after plant has been severed below cotyledons: *a*, four derivatives of single epidermal cell; *e*, epidermis of hypocotyl; *cp*, cortical parenchyma of hypocotyl; *lp*, leaf primordia of adventitious bud.

as many as five or six epidermal cells become meristematic (figs. 29, 30). The identity of the derivatives of an original epidermal cell can be determined by a heavy cell wall about the group, even after four or five subsequent divisions (fig. 30).

When one or two epidermal cells have become active, the under-

lying, adjoining cortical cells lose their chloroplasts, become less vacuolate, and begin cell divisions (fig. 29). These cells continue to divide and enlarge, so that the large intercellular spaces are filled and other adjoining cortical cells in turn become active. The activity of the cortical parenchyma continues until an active zone of cells extends to the endodermis. Growth of the cortical parenchyma results in elimination of the intercellular spaces. These new parenchymatous cells differentiate in place and do not contribute to the elongation of the new axis.

Derivatives of the original epidermal cells by subsequent divisions eventually constitute the axis of the adventitious bud. A dome-shaped group of cells is first formed (fig. 31), and then by rapid cell growth two or three leaf primordia are developed about the periphery of the dome-shaped group of meristematic cells. The first leaf primordia develop rapidly, and the flat group of cells between them later becomes a dome-shaped growing point which is similar to the epicotyl (figs. 32, 33). Vascular bundles are differentiated in the first two or three leaves of the new bud by the time the growing point is well established, and before any vascular system has been established between the stele of the hypocotyl and the new bud. Protoxylem elements are differentiated in the first leaves of the new bud before cell division has begun in the inner layers of the cortex of the hypocotyl (fig. 33). After the cells of the inner cortex have become meristematic, vascular tissue is differentiated progressively inward from the new bud to the endodermis. After many tracheids have been differentiated from the derivatives of the cortical parenchyma, a number of simultaneous tangential and radial divisions take place in the endodermis (fig. 34). Some of the derivatives of the endodermal cells soon differentiate into tracheids, and establish continuity with the tracheids which have differentiated from cells in the pericycle and in the phloem parenchyma (fig. 35). Figure 35 indicates the relative size of the adventitious shoot before vascular connection is established with the vascular elements of the primary axis of the hypocotyl. Very few cells other than elongated parenchymatous cells can be identified as phloem. By the time vascular connection is established with the vascular elements of the hypocotyl, a layer of cells surrounding the tracheids becomes an active cambium.



FIGS. 33, 34.—Longitudinal sections of hypocotyl showing development and differentiation of adventitious buds after plant has been severed below cotyledons: *e*, endodermis.

In a few weeks the secondary tissues resulting from the multiplication and divisions of the cambium of the hypocotyl and those from the cambium of the newly formed shoot develop a plant axis which is almost straight.

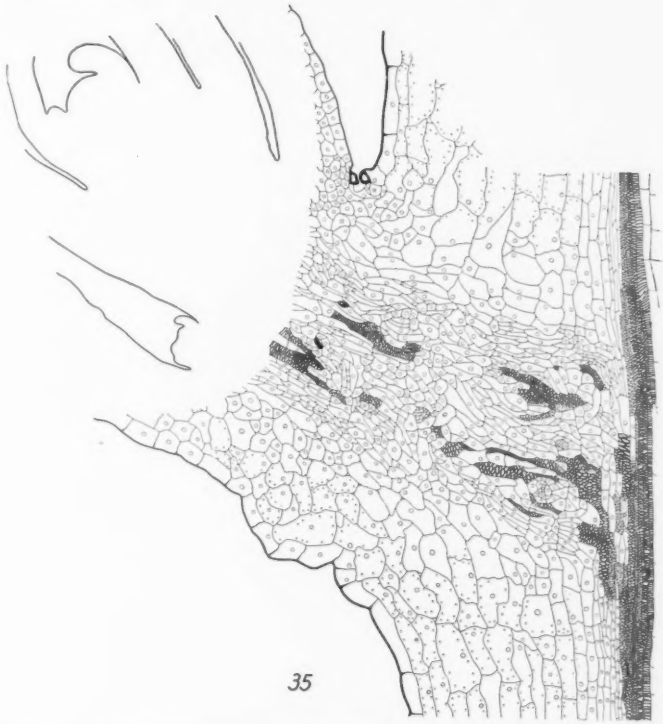


FIG. 35.—Longitudinal section through hypocotyl and an adventitious bud showing differentiation of vascular connection between the new bud and stele of hypocotyl.

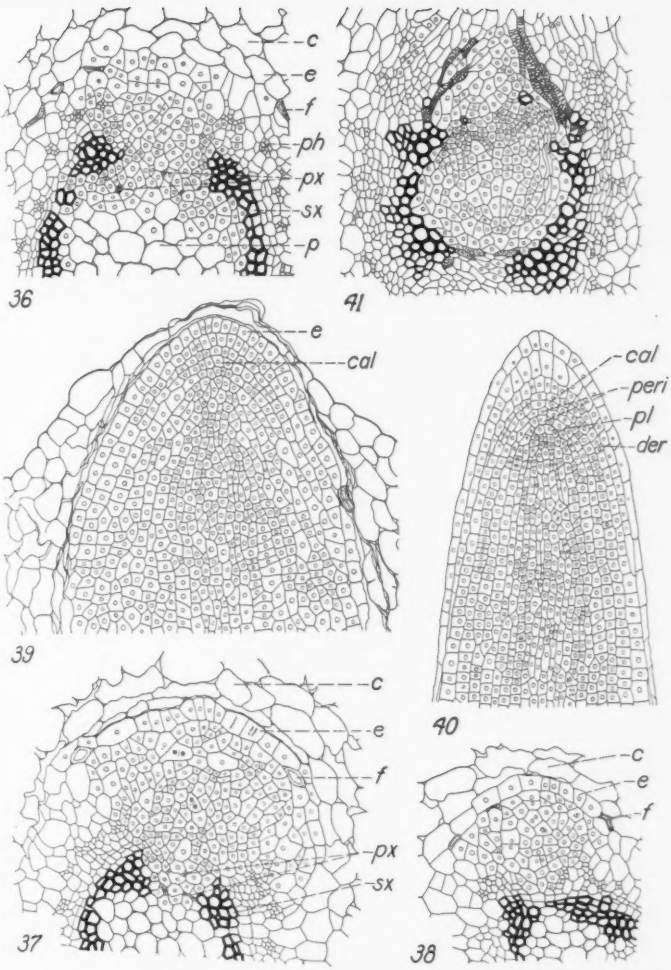
Adventitious roots from hypocotyl

Plants six to ten days old were severed at the middle region of the hypocotyl, and the upper part of the plant set with the hypocotyl in moist soil so that the cotyledons were just above the soil level. Elongation of the hypocotyl soon lifted the cotyledons from 1 to 3 cm. above the ground level, depending on the age of the

plant at the time of cutting. All the cuttings of young plants produced two to five roots along the lower part of the hypocotyl. Roots developed even when the remaining hypocotyl was only long enough to hold the cotyledons in contact. Cuttings of the epicotyl made several nodes above the cotyledons also produced roots.

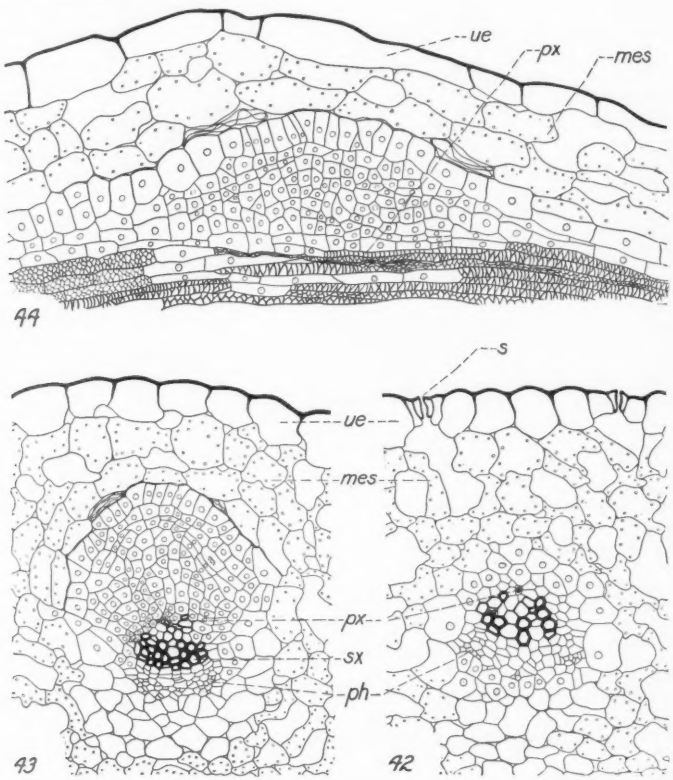
In the hypocotyl a V-shaped ray of parenchymatous tissue extends outward from each protoxylem strand, which is continuous with the protoxylem of the median bundle of a cotyledon, and the adventitious roots are usually initiated by a general activity of the cells in these parenchymatous rays. These roots then come from the hypocotyl in two rows, each of which is directly below the midvein of one of the cotyledons. Often the general activity extends from the outermost layer of the pericycle to and including the parenchymatous cells about the primary xylem (fig. 36). Almost all of the primary xylem is broken down and resorbed so that cell activity involves even the pith. Cell divisions continue in all planes, thus forming a dome-shaped group of cells which pushes outward into the cortex. The endodermis of the hypocotyl can be identified by the early development of pericyclic fibers (1). The endodermis becomes active by cell divisions in a radial plane (fig. 37). This uniseriate layer of endodermal cells keeps pace with the subsequent growth of underlying cells, and forms a layer over the root cap which does not break down until the new root grows 1–2 mm. into the soil. The new root grows by general activity of the cells, with no organized histogens until about the time it pushes through the cortex into the soil. Then at the tip of the new root the cell layer underneath the endodermal layer begins to divide periclinally, thus becoming a true calyptrogen which continues to function as the calyptrogen described for the primary and lateral roots (fig. 39). The periblem and plerome are not definitely established until the root has grown 2–3 mm. into the soil. Figure 40 shows an adventitious root with total length of 2.5 mm., in which the periblem and plerome have become established and are functioning as the histogens described for the primary root.

Cell divisions often occur throughout the pith region of the hypocotyl, and form a solid cylinder of parenchymatous cells which remain active for a month or more after the new roots develop (fig. 41). When two roots develop at the same level on the hypocotyl, the cell



FIGS. 36–41.—Transverse sections of cuttings of hypocotyl showing development of adventitious root. Figs. 36, 37, 39, initiation of roots in ray parenchyma in same radius as protoxylem. Fig. 38, initiation of root in pericycle and phloem parenchyma not in same radius as protoxylem. Fig. 40, median longitudinal section of adventitious root with histogens established (root length 2.5 mm. from hypocotyl). Fig. 41, transverse section of hypocotyl, median through an adventitious root, showing cell activity throughout pith region: *c*, cortex; *e*, endodermis; *f*, pericyclic fiber; *ph*, phloem; *px*, primary xylem; *sx*, secondary xylem; *p*, pith; *cal*, calyptrogen; *peri*, periblem; *pl*, plerome; *der*, dermatogen.

activity of the pith is usually more extensive. The cells of this region of the pith may become differentiated as wound tracheids or remain as relatively thin-walled cells.



FIGS. 42–44.—Figs. 42, 43, transverse sections of cotyledon made at right angles to median vein showing development of adventitious root: *ue*, upper epidermis; *mes*, mesophyll; *px*, primary xylem; *sx*, secondary xylem; *ph*, phloem; *s*, stoma. Fig. 42, first activity of parenchyma surrounding median vein. Fig. 43, early development of adventitious root on adaxial side of median vein of cotyledon. Fig. 44, longitudinal section through median vein of cotyledon showing origin of adventitious root on adaxial side of bundle.

Some adventitious roots of the hypocotyl are initiated by a general cell activity in the pericycle and phloem parenchyma (fig. 38).

In this case they are not in the same plane as the median bundles of the cotyledons. The subsequent development is the same as that described for the roots arising in the plane of the median bundles of the cotyledons.

At the cut surface of the hypocotyl some groups of parenchymatous cells of the cortex, pericycle, phloem, and sometimes regions of the pith divide several times without the derivative cells enlarging. Except in these regions of parenchyma, no extensive wound tissue is formed.

BEALS (3), in describing regeneration phenomena in flax, states: "First the epidermis divides and then the innermost row of those cells and the stimulated cells of the region just beneath form the regenerated part, root or shoot (pl. 16)." In the present investigation the shoots were initiated by the epidermis and the roots from tissues of the stele. Cuttings grown on filter paper in sterile dishes, in the manner described by BEALS, developed roots, all of which originated from tissues of the stele in the manner already described.

Adventitious roots from cotyledons

The cotyledons were severed from seedlings and placed on the surface of moist soil. Some were placed horizontally with the adaxial surface up, others with the adaxial surface down, and others vertically with the cut end set about 5 mm. into the soil. About 90 per cent of the cotyledons taken from plants less than ten days old developed three to six roots, while few of the cotyledons from plants twenty days old produced roots when placed on moist soil. The cotyledons developed an extensive root system and grew to be larger and thicker than those not removed from the axis. Often one to six layers of parenchymatous cells at the cut surface of the cotyledon divided several times without the derivative cells enlarging. Sometimes some of the wound tissue near the larger bundles differentiated tracheids. Cotyledons never produced buds but often lived for more than sixty days, while those on the plant usually live as active photosynthetic leaves for about thirty days.

Usually one root came from near each of the three larger veins of the cotyledon, although as many as three roots frequently developed near one vein. The roots pushed through the adaxial surface

of the cotyledon within 3–4 mm. of the cut end, or emerged from the cut surface. When a cotyledon was placed with the adaxial surface up, the roots either emerged from the cut surface or grew up through the epidermis and then curved over the cut end into the soil.

These roots were initiated by the activity of a layer of parenchymatous cells which surrounds the vascular elements of the bundle (fig. 42). In many cases this layer became active about the entire bundle, and in some cases the phloem and xylem parenchyma also became active. In other cases the activity was limited to the adaxial side next to the xylem. The cell activity usually extended 1 mm. or more along the bundle, and the cambium of the larger veins produced considerable secondary tissue (figs. 43, 44). Increased cell activity on the adaxial side of the vein produced a dome-shaped group of cells (figs. 43, 44) which was either pushed through the upper epidermis or grew about parallel to the bundle. When the roots were 2 mm. or more in length, definite histogens were organized similar to those described for the adventitious root from the hypocotyl.

In two cases the root was organized from wound tissue and grew directly from the cut surface of the cotyledon. All other roots that emerged from the cut surface, or pushed through the epidermis, had their origin in the parenchymatous cells on the adaxial side of a vein at a point 1–4 mm. from the cut surface. From the point of origin the roots either grew through the mesophyll parallel to the vein and emerged through the cut surface or pushed through the epidermis on the adaxial surface of the cotyledon. In a few cases the roots originated almost lateral to a vein but were always slightly nearer the adaxial surface.

Summary

1. Germination of the seed and development of the seedling of *Linum usitatissimum* are described.

2. The primary root is diarch. The growing point has clearly defined histogens. The plerome, periblem, and calyptrogen give rise to the stele, cortex, and root cap respectively. Laterally to the calyptrogen, the dermatogen produces the epidermis by anticlinal divisions.

3. In the development of the vascular elements of the root, two

primary phloem ducts are first differentiated by elongation and breakdown of a single row of cells lying next to the pericycle and alternate with the protoxylem points which are differentiated later.

4. Lateral roots are initiated by regular divisions of some of the cells of the pericycle. One tangential division of these cells of the pericycle forms two layers which in turn divide simultaneously and form four layers of cells. The outer layer later differentiates as the calyptrogen and dermatogen, the next as the periblem, and the innermost two as the plerome.

5. In formation of lateral roots the endodermis becomes active and forms a single layer of meristematic cells overlying the root cap.

6. A simple type of transition from root to cotyledons occurs in the hypocotyl and base of the cotyledons. The primary vascular system of the root, hypocotyl, and cotyledons forms a system largely independent of the subsequent epicotyledonary development. The principal vascular connection of the epicotyl and hypocotyl is by secondary tissues.

7. In the upper region of the hypocotyl most of the primary xylem is broken down by elongation, and is for the most part resorbed by intervening parenchymatous cells.

8. Development of the epicotyl and foliage leaves is described. All vascular bundles of the epicotyl are collateral foliar bundles which do not anastomose but end in the parenchyma between other vascular bundles. The only vascular connection between the primary vascular bundles of the stele is by a continuous vascular cylinder of secondary tissues which is formed by the activity of fascicular and interfascicular cambium.

9. The basal part of seedlings severed at the middle region of the hypocotyl regenerated five to twenty buds, of which usually only one continued development and became a shoot. Regenerated buds originated by successive division of epidermal cells. Vascular connection between the primary stele of the hypocotyl and the new shoot was initiated by renewed cell activity of cortical parenchyma, endodermis, pericycle, phloem parenchyma, and cambium, in the order named. Tracheids of the vascular system were first differentiated in the leaves of the new bud, and then differentiation occurred progressively inward to the stele of the hypocotyl until con-

tinuity was established between the tissues of the new shoot and the stele of the hypocotyl.

10. Seedlings severed in the middle region of the hypocotyl and the upper parts used as cuttings set in moist soil or placed in moist petri dishes produced adventitious roots from the hypocotyl. These roots originated from parenchymatous tissue which in the same root often involved pericycle, phloem, and pith. The endodermis of the hypocotyl became active and produced a single layer of cells overlying the tip of the developing root.

11. Cotyledons cut from seedlings and placed on moist soil produced adventitious roots and lived for more than two months. These roots originated from a layer of parenchymatous cells on the adaxial side of the larger veins. In no case did these cotyledons produce shoots.

The writer wishes to express grateful appreciation to the members of the Department of Botany of the University of Chicago for helpful suggestions and criticisms during the progress of this investigation.

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A NEW OCCURRENCE OF THE OLDEST KNOWN TERRESTRIAL VEGETATION, FROM BEARTOOTH BUTTE, WYOMING

ERLING DORF

(WITH PLATES V, VI)

Introduction

Although the widespread and highly organized vegetation of the upper Paleozoic era (Upper Devonian and Carboniferous periods) is profusely represented in many localities throughout the world, the primitive plants of the Lower Devonian, which are the oldest terrestrial forms thus far known, are recorded from only a few scattered localities, chiefly in western Europe, Australia, and eastern Canada. In western North America the whole of the Paleozoic system is deficient in remains of land plants, chiefly because most of the sediments are of marine origin. It is therefore of interest to report the discovery of a small but well preserved Lower Devonian flora from an estuarine deposit at Beartooth Butte, Wyoming.

The plant remains were discovered and collected during the summer of 1932 by a party of investigators including the writer, in connection with the Princeton Research Project in Montana and Wyoming. The expedition, primarily concerned with the collection of primitive fossil fishes, was conducted under the auspices of the Princeton Scott Fund, to which the writer expresses his grateful appreciation.

Occurrence

The plant impressions were found in a lens of impure limestone which outcrops on both the eastern and western cliff faces of Beartooth Butte. This butte is situated in the northern part of Park County, Wyoming, 3 miles south of the Montana line, and is an outlier of Paleozoic sediments resting on pre-Cambrian granites and gneisses. The limestone lens, which appears to be an ancient channel deposit, 175 feet thick and 2000 feet across, lies, with erosional unconformity, on the marine Bighorn dolomite of Ordovician age, and

is in turn overlain, with less marked unconformity, by the marine Jefferson limestone of the Middle Devonian. A preliminary study of the red and gray impure limestone of the lens seems to indicate deposition in a quiet, shallow, drowned valley along a submerging coast line. Absence of peaty layers suggests that swampy conditions did not prevail, and that the plants grew as a fringe around or possibly in the shallow borders of the basin.

In direct association with the plant remains occur numerous well preserved fish plates. A preliminary report (4) on the fish fauna collected during the summer of 1931 indicated an abundance of Ostracoderms and Arthrodires. The assemblage is clearly of Lower Devonian aspect.

The plant fossils were collected partly *in situ* and partly from large talus blocks on the steep slopes below the outcrop. Unfortunately no petrifactions were discovered, so that very little is as yet known regarding the detailed internal structure of the plants. Attempts to study the microscopic details of the impressions by the film-transfer or collodion film-pull methods, or by maceration in acids, have thus far been unsuccessful, owing to the fact that very little carbonaceous material has been left adhering to the rock matrix.

Description of species

The descriptions are based entirely on the megascopic details. It is likely that if better material is procured from this locality, microscopic study will warrant more definite determinations. However, five distinct types are described and discussed in detail:

1. *Psilophyton wyomingense*, new species: flattened spiny branch systems.

2. (*?*) *Psilophyton* sp.: an elongated fructification (?).

3. *Bucheria ovata*, new genus and new species: narrow axes with clustered, terminal sporangia (?).

4. *Hostimella* sp.: flattened branch systems without spines.

5. (*?*) *Bröggeria strobiliformis*, new species: cylindrical fructification of strobilus-like nature.

All of these forms are referred to the primitive order Psilophytales. The generic determinations of types 2 and 5 are unfortunately tentative and somewhat questionable, because of scarcity of material in

the collections. The remaining three types are each represented by twelve or more well preserved specimens.

PSILOPHYTON Dawson

Psilophyton wyomingense n. sp. (figs. 2–6, 8)

DESCRIPTION.—Somewhat flexuose branch system with main axis and subequally dichotomous lateral branches, probably sympodially formed. Lateral branches given off from main axis at acute angles and repeatedly bifurcating dichotomously at similar low angles, apparently in one plane. Surfaces irregularly and obscurely marked with rounded aréoles, presumably spine scars, and with occasional fine longitudinal striations. Entire branch system bearing closely set spines which are irregularly disposed and more numerous on main axis. As seen in profile, spines are of various lengths up to 2 mm. and are extremely fine, tapering from an only slightly enlarged basal portion to a pointed distal end.

DISCUSSION.—This species is represented in the collections by numerous flattened, spinous branch systems whose characteristics are so closely comparable with those of previously described and figured species of *Psilophyton* that its reference to that genus is unquestionable. It is indeed difficult to differentiate this form from the original species, *P. princeps* Dawson, even in its narrowest sense (=var. *ornatum*) as redefined by HALLE (**10**) and WHITE (**30**).

A comparison with DAWSON'S original figures of the typical spine bearing branches of *Psilophyton princeps* (**7**) brings out the following differences which seem to necessitate defining the Wyoming form as a separate and distinct species: (1) lateral branches are more repeatedly bifurcated, unlike those of *P. princeps;* (2) branch tips do not show the characteristic circinate habit of vernation; (3) spines are finer, more closely spaced, and do not enlarge to so wide a base as in DAWSON'S specimens. The same general similarities and specific differences are apparent in comparisons with other figured specimens of the spiny branches of *P. princeps* from France (BERTRAND **2**), Scotland (LANG **20**), and Norway (HALLE **10**). The long fine spines of our species agree more closely with those of *P. goldschmidtii* Halle from Norway (**10**). In this latter species, however, the main axis is considerably larger than the lateral branches, which bifurcate at

wider angles; also the spines are much less numerous on the thicker branches and virtually absent in the thin laterals. These differences, which are somewhat less conspicuous in comparison with the German forms referred to this species (**15**), would seem sufficient to warrant a new specific determination for the Wyoming form.

A striking similarity to the Wyoming type is seen in the single species recorded from the archipelago of Bulandet in western Norway (**26**). Unfortunately this form was designated only as *Psilophyton* sp., although HALLE (**10**) regards it as a typical representative of *P. princeps*. More recently KRÄUSEL and WEYLAND (**13**) have intimated that it may well be a thin branch of *Asteroxylon elberfeldense*.

At Beartooth Butte, in the absence of more complete specimens of the entire plant, there remains the possibility that the forms here referred to *Psilophyton wyomingense* may actually be the outer branches of a larger plant whose basal portions have not yet been found. Spiny stems, formerly referred to *P. princeps*, have recently been found in direct connection with larger stems of the *Thursophyton* type in Middle Devonian beds in Germany (**13**). These are referred to the species *Asteroxylon elberfeldense*, whose morphological characters are now almost completely known. Until entire structures are found, however, it seems best to make no assumptions regarding the basal portions of our species and to retain the generic name *Psilophyton*.

In this connection it is possible that the specimen shown in figures 4, 5, and 8 may represent the larger basal branches of our species. The figures show the spiny branches arising almost perpendicularly from one side of a somewhat larger spiny stem (rhizome?). The fine, pointed spines, the numerous spine scars, the low-angled dichotomous branching, and the faint longitudinal striations are all characters possessed in common with the type specimen just described. The writer therefore feels justified in suggesting that this specimen can be referred to *Psilophyton wyomingense* in spite of the absence of an actual connection with the type of the species. A possible basal portion with such truly *Psilophyton*-like characteristics suggests that the *Thursophyton* type of larger branches did not exist in the Wyoming flora.

It is unfortunate that little is as yet known regarding the finer branches and possible fructifications of this species. The thin

axis shown in figure 6 possesses essentially the same characters as the lateral branches of the type specimen, being only somewhat thinner and less spinous. The more widely spaced and less numerous spines agree with other figured specimens of the outer branches of *Psilophyton* (HALLE **10**, LANG **20**). It is possible that some of the very thin dichotomous branches in our collection which are entirely lacking in spines may represent the extreme ends of the spiny types. The fructifications, still unknown in connection with spiny axes, may possibly be the form to be described later as (?) *Psilophyton* sp., which is similar in size and shape to the fructifications of *Psilophyton* described from elsewhere. For that matter it is not at all certain that the narrow spineless axes bearing clustered fertile organs, described here as *Bucheria ovata*, may not have been the outer branches of the spiny stems of *Psilophyton wyomingense*.

In summation, the Wyoming specimens are clearly comparable with the typical branch systems found elsewhere in Devonian rocks and referred to the genus *Psilophyton*. While described as a new species whose entire growth form is at present incompletely known, a close similarity is suggested with the more authentic forms of *P. princeps* and *P. goldschmidtii*. In the most recent contribution to the study of Lower Devonian floras, LANG (**20**) emphasizes the fact that in the re-examination of the floras of the Lower Old Red of Scotland it is impossible to make a clear specific distinction between specimens of the *P. princeps* type and those of the *P. goldschmidtii* type. He prefers to consider the latter as a form of the more comprehensive species *P. princeps*.

(?) **Psilophyton** sp. (fig. 1)

Only a single specimen of this type has been found at Beartooth Butte. It is doubtfully referred to *Psilophyton* until such time as material may be found to enable a more accurate determination. As seen in figure 1, the specimen is an elongate, linear, pointed body which resembles in size and shape the spore cases borne individually on the terminal ends of branches of the *Psilophyton* type. Spore cases from eastern Canada figured by DAWSON (**7**) and by LANG (**19**) are of essentially the same character as the single specimen here shown. Other closely comparable forms are those from the Lower

Devonian of Matringhem, northern France (2), and those referred to *Dawsonites arcuatus* Halle from Norway (10).

Bucheria, n. gen.

DESCRIPTION.—Spineless, narrow axes bearing at their distal ends clusters of small rounded appendages, which are bilaterally-symmetrically arranged on opposite sides of the axes. Appendages closely set, sessile, and opposite to subopposite.

Bucheria ovata, n. sp. (figs. 9–17)

DESCRIPTION.—Long, narrow axis bearing at distal end, in two rows, clusters of small ovate bodies, symmetrically arranged on opposite sides of axis. Axis averaging 1.8 mm. in width and up to 6.5 cm. in length, non-spinous, and marked by a few pronounced longitudinal costae and numerous faint interrupted striations. Lateral bodies up to sixteen in number, closely set, sessile, and somewhat pointed at their distal portions. Dorsal and lateral views of these bodies often showing longitudinal median splits extending from their central areas to their pointed ends. Microscopic structural details unknown.

DISCUSSION.—This species, which is represented in the collections by twelve or more well preserved specimens, is at present restricted to what are undoubtedly the fertile portions of a plant whose vegetative parts are as yet unknown. Although proof is still lacking, it is reasonable to suppose that the impressions are of leafless axes bearing terminal spikes of sporangia. Innumerable attempts to study the microscopic details by the film-pull or film-transfer methods have been unsuccessful, so that their sporangial nature is still an assumption based on their general appearance and on comparisons with European and Australian forms in which spores have actually been found (22).

As seen in figures 14–16, there is in several of the bodies a clear distinction between an outer rim and an inner, solid structure. The solidity and distinctness of this central area are due to sediment which apparently filled in a hollow cavity at the time of preservation. That it had previously contained spores is suggested as a possibility. The outer layer may be a thickened epidermis. In a recent

letter from Dr. LANG, he states that film-pulls made on two specimens sent to him "have yielded appearances suggestive of a distinction of wall and spores, but only suggestive."

A somewhat more convincing detail is the definite split clearly seen in the dorsal and lateral views of several specimens (figs. 9–12, 15, 16). In a few of the forms, particularly figure 15, this split is seen to continue to the distal end where it divides the structure into two bluntly pointed lobes. This type of split may well have been the line of dehiscence in the individual sporangia, in which case the inclosed spores had undoubtedly already been liberated before preservation.

The absence of radial symmetry in this species suggests a dorsiventral arrangement of the sporangia on their axes. Since dorsiventrality is normally found only in plagiotropous (oblique or horizontal) portions of plants, it seems plausible to regard these impressions as fertile leaves, or sporophylls, rather than as central spikes, in which radial symmetry would be present. This observation intimates that the axes are rachises of divided fronds rather than of stem nature.

In the comparison of *Bucheria ovata* with other Devonian fructifications, none has been seen which possesses characters similar enough to warrant specific, or even generic, identity. One form referred to as ? *Dawsonites* sp., from the Lower Devonian of Willwerath, Germany (16), might possibly belong to the same genus. The figured specimen (16, text-fig. 52) shows a narrow axis terminated by two definite rows of oval, elongated organs (spore cases ?) and a possible third row indicated by one of the bodies assuming a central position between the lateral rows. It is reasonable to suppose from the slight displacement of several of the bodies that this single erratic one might well have been shifted out of its normal position in the process of preservation. If that be true, as seems likely, it would be better to place both the Wyoming and the German forms in the new genus *Bucheria*. The genus *Dawsonites*, to which the German specimen is questionably referred, is defined by HALLE (10) as having dichotomously dividing curved branches bearing single terminal spore cases which are non-sessile and narrowly obovoid or short fusiform.

None of these features is evident in *Bucheria ovata* or the German ? *Dawsonites* sp.

In addition to ? *Dawsonites* sp., the Wyoming form also presents a fair comparison with the "cones" of *Arthrostigma gracile* from the Lower Devonian of Campbellton, Nova Scotia (**8**, pl. XXIV). As figured, this specimen seems to have been incompletely and poorly preserved, showing only the two rows of crescent-shaped bodies without indicating their connection with an axis or their morphological details. DAWSON (**8**) intimates that they may have been "spikes of sac-like spore cases."

From the Lower Old Red of Scotland, *Zosterophyllum myretonianum* Penh. shows a somewhat similar morphological construction (LANG **18**). This species has clustered, terminal spore cases differing from the Wyoming types mainly in their reniform shape, radial arrangement, and wider spacing on their axes. *Z. australianum* L. & C. (**22**) from the Lower Devonian of Australia is equally similar in general composition. The resemblance of our form to both these species of *Zosterophyllum*, in which actual spores within the appendages have been definitely demonstrated (**22**), is particularly significant in intimating the sporangial nature of the appendages in *Bucheria ovata*. Another species based on clustered, terminal sporangia is *Hostimella racemosa* Lang from the Middle Old Red of Scotland (**17**). While this also is comparable with *Bucheria ovata*, it differs considerably in that its spore cases are stalked, more pointed, and much more widely spaced on the axes.

In the absence of a definite connection between the terminal clusters of sporangia here referred to as *Bucheria ovata*, and larger branches or stems, it is impossible to ascertain just what the vegetative portions of this plant looked like. The spineless nature of the long narrow axes bearing the fructifications suggests a connection with the naked stems described in a following paragraph as *Hostimella* sp., although there remains the possibility that they may belong to *Psilophyton wyomingense*, whose end branches might have been spineless. That this view is not a far-fetched probability is shown by the discovery of naked spore-bearing branches at the ends of spiny stems of the *Psilophyton* type in *Asteroxylon elberfeldense* from

the Middle Devonian of Germany (13). On the other hand, the presence in the Beartooth flora of a sporangium (?) of the *Psilophyton* type (discussed here as ? *Psilophyton* sp.) makes it more plausible to assume that this type of single fructification was borne by the spiny branches of *P. wyomingense*, and that the clustered spore cases (*Bucheria ovata*) were developed on branches of another type, perhaps *Hostimella* sp.

The preceding description and discussion leave much to be learned as to the true nature of these clustered fructifications. Despite the lack of more complete material, the many well preserved and well defined specimens in the collections seem to justify the new generic name *Bucheria*. This designation is intentionally noncommittal as to systematic affinities, which are at present doubtfully known. It is with pleasure that the writer has named the genus in honor of Dr. and Mrs. WALTER H. BUCHER, of Cincinnati, Ohio, who were members of the 1932 expedition to Wyoming.

HOSTIMELLA P. & B.

Hostimella sp. (figs. 18, 19)

The collections from Beartooth Butte contain about twelve thin, dichotomously branched stems without spines. Similar forms are recorded from most of the Devonian localities elsewhere and are usually referred to the form type, *Hostimella* sp., although there is no very definite line of demarcation between this designation and *Aphyllopteris* (22). Included as *Hostimella* sp., however, are two types of smooth axes differing chiefly in the presence or absence of peculiar axillary "budlike" structures. These are to be seen in several specimens from the Middle Old Red of Scotland (17), the Walhalla series of Australia (22), the Middle Devonian of Germany (12), Belgium (5), and Bohemia (27). In none of the Wyoming specimens is there any trace of an "axillary bud." With the exception of such forms as do possess these peculiar structures, it is difficult to distinguish the Wyoming specimens from most of the figured specimens of *Hostimella* sp., from either the Lower or Middle Devonian. Those recorded by HALLE (10) from the Lower Devonian of Norway are perhaps the most strikingly similar.

Only recently has a little light been cast on the systematic position of these common Devonian forms. Naked, dichotomous branches, formerly referred to *Hostimella hostimensis* P. & B., have been found in direct connection with spiny stems of the *Psilophyton* type in Germany; the complete plant is now known as *Asteroxylon elberfeldense* Kr. & W. (13). This does not mean, of course, that all such branches found elsewhere must necessarily have been connected with spiny stems, but at least it presents that possibility as more than an unfounded assumption. In the Wyoming forms there is some reason to believe that this was not the case; a few of the naked branches are fully as thick as those of *Psilophyton* bearing many well defined spines; also the thinner branches without spines are not smaller than those of *Psilophyton* which do bear spines.

It is obvious that these naked branches must remain as of unsatisfactory classification, despite their common occurrence in Lower and Middle Devonian floras, until such time as they may be found in direct association with larger stems of similar or different characteristics. In the present flora no such complete specimens have been found.

That the terminal ends of the naked branches of *Hostimella* sp. might have borne the fructifications described as *Bucheria ovata* has been mentioned in the discussion of the latter species.

Bröggeria Nathorst

(?) **Bröggeria strobiliformis** n. sp. (fig. 7).

This species is represented in the Wyoming collections by only a single specimen. In this, however, the characters are well defined and of a decidedly new type, worthy of recognition as a matter of record.

As shown in figure 7, the species is based on an impression of a cylindrical, strobilus-like body studded with numerous small, rounded bodies. It resembles in general appearance the terminal, sporangia-bearing catkins of Equisetaceous types. Until much more material of this type may be discovered and studied, the determination must remain as unsatisfactory and questionable.

The tentative reference to *Bröggeria* is based on comparisons with

a species recorded from the Lower Middle Devonian of western Norway (**26**), but the similarity is none too great. The writer feels certain that the Wyoming form cannot be definitely referred to any previously recorded genus. It would seem unreasonable, however, to describe a new genus on such insufficient data as our single specimen affords.

Bröggeria norvegica Nat., as figured on plates 3 and 4 (**26**), is clearly of strobiloid nature. It is considerably larger than the Wyoming type and exhibits a much more open and loose arrangement of sporangia (?). It also possesses short, leaflike appendages which separate the individual bodies from one another. This feature is not evident in our specimen. In general appearance, however, the Norwegian species is the only Devonian form with which a comparison is at all possible.

Correlation

In attempting to determine the exact age of the plant-bearing beds at Beartooth Butte, several significant factors must be kept in mind: (1) Comparisons must of necessity be with floras which are far distant from the Beartooth flora; such long-distance correlation is not entirely satisfactory. (2) The Beartooth flora, consisting of only five distinct forms, is too small for accurate comparison with larger floras elsewhere. (3) None of the species is identical with any recorded from other localities, so that conclusions must be drawn from index types rather than from index species. (4) The exact ages of the Canadian and European Devonian floras, with which comparisons are made, are by no means definitely settled. Obviously, unless the stratigraphic position of such floras is determined by superposition and associated animal remains, the exact age references cannot be made until the Devonian floras are better known.

Fortunately the age determination of the Beartooth beds is helped by the stratigraphic relations. Unconformably below the beds lies the Bighorn dolomite of Upper Ordovician age (MILLER **25**); above lies the Jefferson limestone of the Middle Devonian (LOVERING **24**). We are thus clearly dealing with a formation of either Silurian or Devonian age. The possibility of Silurian age is extremely remote; no authentic terrestrial floras have thus far been recorded from Silurian rocks anywhere in the world. The fish fauna which occurs

in direct association with the plant remains is distinctly of Lower Devonian aspect (BRYANT 4). Evidence will be presented to indicate that the plant remains substantiate the age reference based on the fish remains.

Considering the Beartooth flora as a whole, it is apparent that we are dealing with a representative of the so-called *Psilophyton* flora (ARBER 1) of the early Devonian. This type is replaced in the upper part of the Middle Devonian by the well known and more highly organized *Archeopteris* flora (13). It is thus relatively certain that the Beartooth flora, aside from stratigraphic and other paleontologic implications, must be older than the Upper Middle Devonian.

Among the European representatives of the *Psilophyton* flora, the association of *Psilophyton goldschmidtii*, *Hostimella*, and ? *Dawsonites* sp. from the Lower Devonian of Willwerath, Germany (KRÄUSEL and WEYLAND 16) is of essentially the same character as the Beartooth flora. The three Willwerath types are among the most similar correlatives of the three most abundant Beartooth species, *Psilophyton wyomingense*, *Hostimella* sp., and *Bucheria ovata*, as previously stated. Other floras which are comparable with the Beartooth flora as an association of types are the Campbellton of eastern Canada (7), the Lower Old Red of Scotland (20), and the Röragen of Norway (10), all of which are considered of Lower Devonian age. While these observations tend to substantiate the evidence of the associated fish remains and the stratigraphic position for a Lower Devonian age reference, it may be instructive to compare the individual forms in the Beartooth flora with related forms elsewhere.

Psilophyton wyomingense is well represented in the Beartooth collections. As already pointed out, its nearest relatives, as judged by close similarity in megascopic characters, are *P. princeps* and *P. goldschmidtii* (both of widespread distribution), and *Psilophyton* sp., from Bulandet, Norway. The typical spine-bearing stems of *Psilophyton* are especially characteristic of the Lower Devonian (10), as exemplified by their occurrence at Campbellton (7), Matringhem (2), Röragen (10), and in Scotland (20). LANG (17) reports that authentic specimens of *P. princeps* are known only from the Lower Old Red of Lower Devonian age, although the "name has frequently been loosely applied to remains" in the Middle Old Red of Lower to Mid-

dle Devonian age. *P. goldschmidtii* is confined to the Lower Devonian and is considered to be one of the most widespread Lower Devonian species of Europe (15). The third correlative, *Psilophyton* sp., from Bulandet, Norway, is likewise referred to the Lower Devonian (10).

Bucheria ovata, which is also abundant in the Beartooth flora, is of the same general construction as ? *Dawsonites* sp., the "cones" of *Arthrostigma* and *Zosterophyllum*. The described and figured specimen of ? *Dawsonites* sp. is of a type which is recorded from only one European locality; the beds in which it occurs are referred to the Lower Devonian (16). In eastern Canada, the "cones" of *Arthrostigma* are likewise confined to the Lower Devonian (8). The same age determination is given for the beds containing *Zosterophyllum myretonianum* (18) and *Z. australianum* (22).

Hostimella sp. possesses less distinctive characteristics, which detracts from its usefulness as a guide fossil. In all of the Beartooth specimens, however, there is a notable lack of the small "axillary buds" observed in *Hostimella* sp. from several European localities. LANG and COOKSON (22) have pertinently pointed out that these axillary budlike structures are not known in the *Hostimella* branches of the Lower Devonian in Europe but are recorded from several scattered horizons in the Middle Devonian. Since this publication, "axillary buds" have been found in the Lower Devonian of Scotland (20), but are not abundant and cannot be considered as characteristic. While such negative evidence is of doubtful value when considered alone, it does afford additional corroboration of the Lower Devonian age reference indicated by *Psilophyton wyomingense* and *Bucheria ovata*.

The two forms (?) *Bröggeria strobiliformis* and (?) *Psilophyton* sp. are represented in the collections by only one specimen each, neither of which is considered distinctive enough to render a definite generic determination. They are consequently of little or no value as indices of geologic age.

From the foregoing discussion it seems apparent that the combined evidence derived from the plant remains, the associated fish remains, and the geologic relations of the fossiliferous beds is entirely indicative of a Lower Devonian age reference.

Botanical considerations

Perhaps the most significant result of the Beartooth Butte discoveries is the extension into western North America of the early Devonian *Psilophyton* flora, which was apparently more widespread and uniform than might have been suspected.

It is also becoming more evident that the spiny branch system of *Psilophyton princeps* and its close relatives were among the most widespread of the Lower Devonian forms in the northern hemisphere, and that the clustered spore cases represented by *Bucheria ovata* were by no means as limited as is indicated in the European record.

The chief botanical interest in the Beartooth flora unquestionably lies in the questions which it raises regarding the true nature of these simple, ancient plants. That they were truly terrestrial is at present merely a deduction based on their similarities to European forms in which vascular tissue, cuticularized epidermis, and stomata have been shown to be present by microscopic studies. It is possible that the faint, longitudinal striations on many of the Beartooth branches may be due to the elongated cells of a hypodermal layer, but as yet no positive evidence of structure of any kind has been obtained.

While discussions continue as to the true systematic affinities of these primitive Psilophytales, it is impossible to trace the ancestry of the more highly organized phyla of later eras. Although the Psilophytales are generally considered to be the simplest and most primitive of the pteridophytes, ARBER (**1**) regarded them as highly advanced terrestrial thallophytes, and CHURCH (**6**) placed *Hornea*, a typical Psilophytalean, in the bryophytes. Nor can we be certain, as SCOTT (**28**) has pointed out, that their simplicity was wholly primitive and ancestral; perhaps unfavorable conditions of growth had already at this early date produced a certain amount of reduction. While such a possibility may apply to the fossiliferous peat deposits of Scotland, there is no geologic evidence to intimate that the Beartooth flora grew in an abnormal or unfavorable habitat. That the spines of *Psilophyton*, moreover, were emergences and not reduced leaves has been suggested by their irregular disposition and the diversity in size in any given area (**10**); more recent microscopic investigations (**9**, **19**) have substantiated such suggestions and have indicated that the spines were probably of secretory glandular nature.

There seems to be a growing conviction, corroborated by increasing evidence, that the Microphyllae and Macrophyllae, and probably also the Articulatae, can be traced back to the Psilophytales (11). In the Beartooth flora only microphyllous forms are found, unless the dorsiventral symmetry of the axes of *Bucheria ovata* and *Hostimella* sp., and the blunt tapering (intimating a limited growth) of such axes might be regarded as suggestive of megaphyllous rachises (10). It is possible that the dorsiventral sporophylls (*Bucheria ovata*) may be of the type which in the course of descent could have become sterile and have developed into broader foliage leaves.

The problem of the relation of radial to dorsiventral symmetry is by no means elucidated by the Beartooth discoveries. BOWER (3) believes that radial symmetry in the sporophyte is primitive and dorsiventrality a secondary condition. LIGNIER (23) and TANSLEY (29) adopt the opposite view. In *Bucheria ovata* the symmetry is distinctly dorsiventral; similar types of fructifications from other horizons of the same age present a definite radial symmetry, however, leaving the question of the ancestral type unsettled. In the dorsiventrality of *B. ovata*, however, there is a possibility of relationship to the primitive Filicales, in which dorsiventrality is the normal symmetry, as contrasted with the usual radial symmetry of the Lycopodiales, Sphenophyllales, and Equisetales.

It is interesting to note that a comparison of the clustered fructifications of *Bucheria ovata* with herbarium material at the New York Botanical Garden brought out a general similarity in morphological construction to several of the simple ferns, such as *Botrychium* and *Ophioglossum*. While the writer feels that the Lower Devonian floras are far too distantly removed in time from our present flora to attempt to establish systematic affinities, the comparison at least indicates the presence in the Lower Devonian of a type of fructification which is not radically different from the arrangement in several simple, modern ferns.

With respect to the higher groups of plants there is in (?) *Bröggeria strobiliformis* a suggestion that the Equisetales are represented in the Beartooth flora; this species is based on the small, strobiloid catkin whose appearance is essentially as in the Equisetales. More and

better material will have to be found, however, before this suggestion, as well as others, can be interpreted as a definite indication of relationship.

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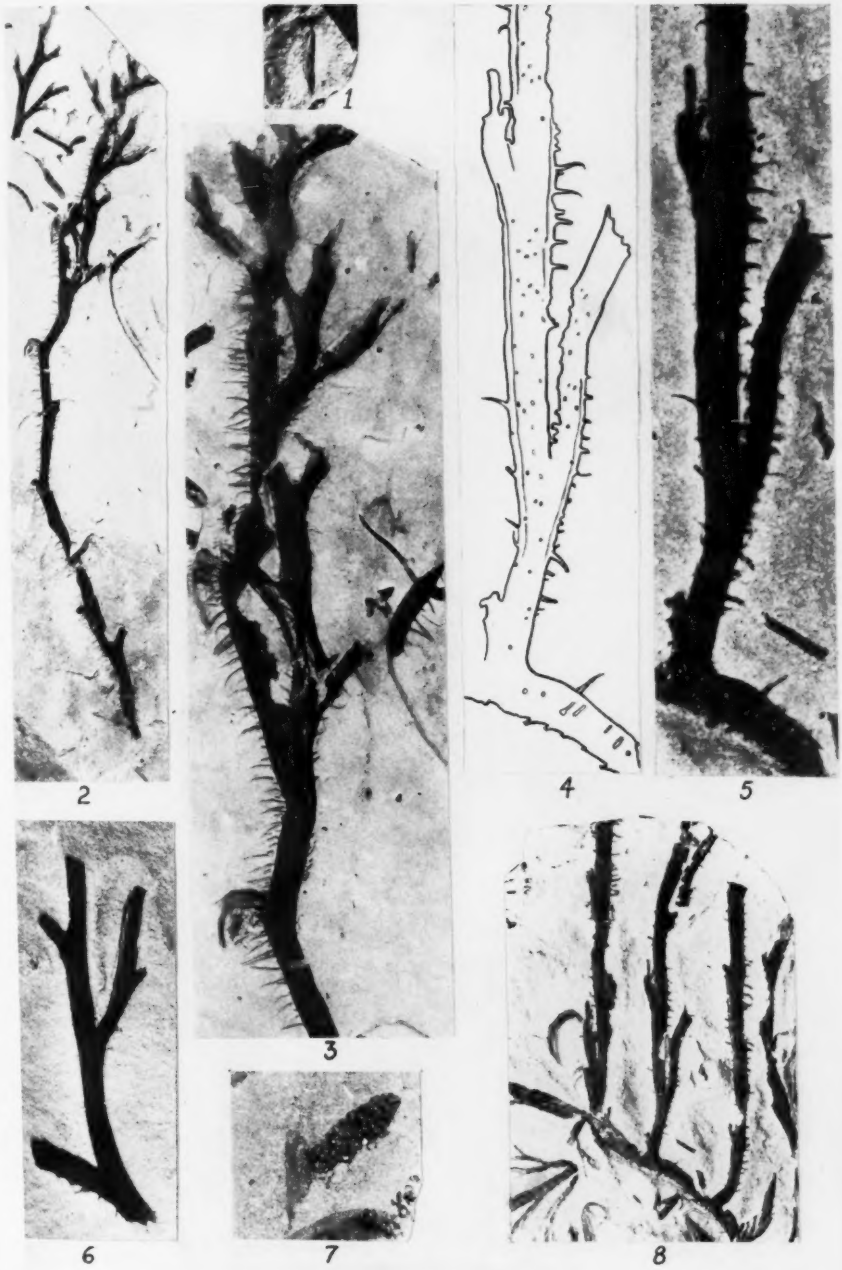
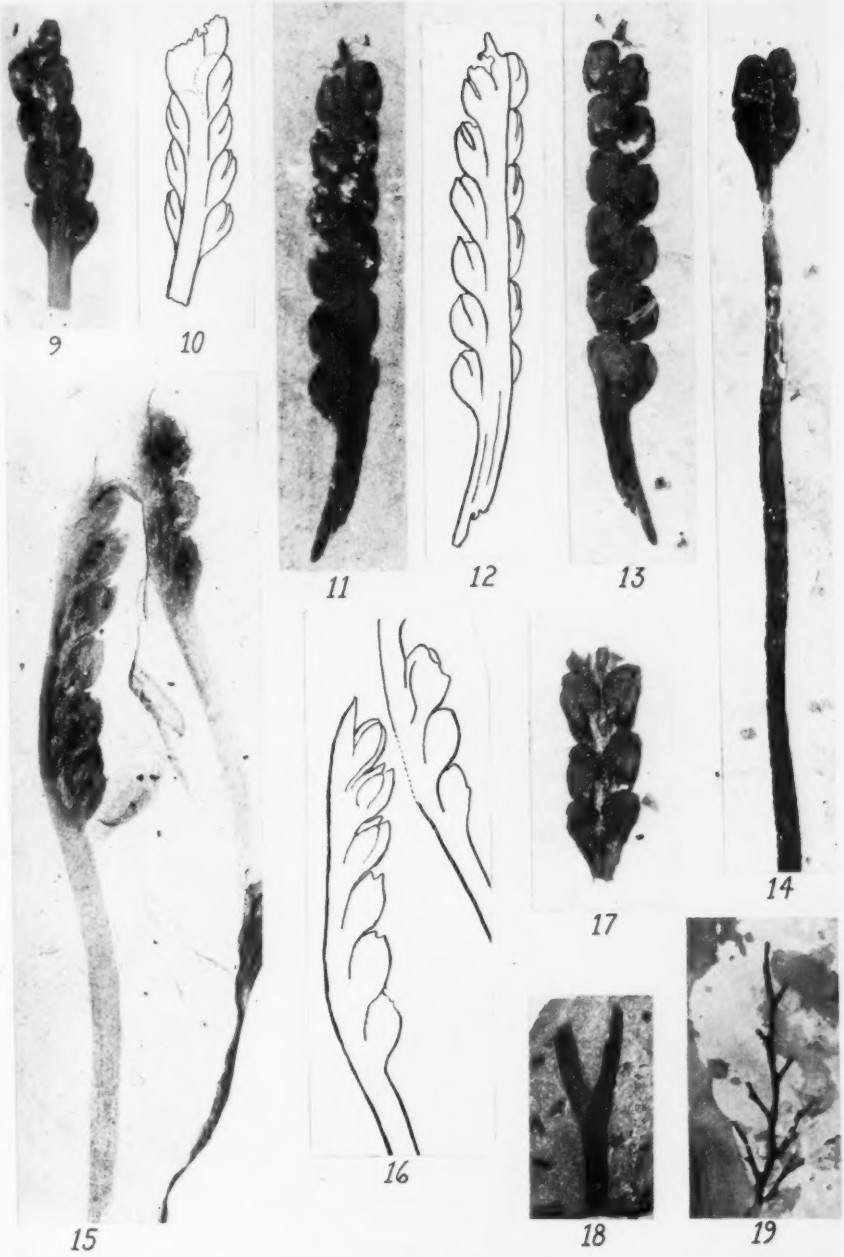


DORF on EARLY DEVONIAN



DORF on EARLY DEVONIAN

EXPLANATION OF PLATES V, VI

PLATE V

FIG. 1.—(?) *Psilophyton* sp., a single detached spore case (?); × 0.833.

FIGS. 2, 3.—*Psilophyton wyomingense*, type specimen, showing mode of branching and numerous spines. Fig. 2, × 0.833; fig. 3, × 2.5.

FIGS. 4, 5, 8.—*P. wyomingense*, showing typical spiny branches with faint longitudinal striae and prominent scars; upright position of branches suggests that the oblique branch may be a rhizome. Figs. 4, 5, × 2.5; fig. 8, × 0.833.

FIG. 6.—*P. wyomingense*, showing only few small spines on otherwise naked, outer branch. × 4.58. (Photograph by W. H. LANG.)

FIG. 7.—(?) *Bröggeria strobiliformis*, showing small rounded spore cases (?) in a catkin. × 2.5.

PLATE VI

FIGS. 9–17.—*Bucheria ovata*, fructification consisting of ovate spore cases (?) in terminal cluster; figs. 9, 10, dorsal views showing medial splits; figs. 11–13, type specimen and its counterpart showing both dorsal and ventral impressions; fig. 14, broken cluster showing long spineless axis; figs. 15, 16, lateral views of two incomplete clusters showing distal splits and prominent central area; fig. 17, ventral view. × 2.5.

FIGS. 18, 19.—*Hostimella* sp., showing dichotomous branches without spines. × 0.833.

SOME PHYSICO-CHEMICAL PROPERTIES OF SEED EXTRACTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 447

CHARLES A. SHULL AND JOHN W. MITCHELL

(WITH ONE FIGURE)

Introduction

Following the processes of fertilization of the egg and polar nuclei in the embryo sac, the development of the embryo and its associated reserve food tissues is marked by the rapid accumulation and condensation of the food materials translocated to the ovule. Soluble salts either come from the soil solution directly, or they are withdrawn from other regions of the plant where these salts have been temporarily in use or from regions undergoing senescent degradation. Soluble organic constituents come from the regions of manufacture, or from tissues in which temporary storage has taken place in anticipation of the needs of the embryo. These substances furnish the building material for the permanent cellular structures of the embryo itself, and for the deposition of its food supplies either within its own cells or in adjoining or surrounding tissues. Because the physiological processes involved during development of the seed are predominantly condensations, the embryo and its food reserves are made up very largely of relatively insoluble compounds, polysaccharide carbohydrates, oils and fats, proteins, protoplasm, and salts of these organic substances or their chemical relatives.

The translocation forms of all of these substances are soluble compounds. As these soluble constituents reach the centers of use, and are converted into insoluble tissue components and food reserves, the osmotic concentration of the cell sap of the embryonic and endosperm cells is kept low. Indeed it is possible that this maintenance of low osmotic concentrations in the region of building and storage is an important means by which the embryo makes its demands upon

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

the remainder of the plant body for its necessary supplies of building materials. For, as MASON and MASKELL (3) have shown, translocation usually occurs in a direction correlated with general osmotic gradients. This, however, should not prevent us from recognizing the possibility, or even probability, that the plant can, and perhaps often does, move its soluble materials in directions contrary to osmotic gradients.[2] Even when condensation does not go far enough to produce actual insolubility, as in the formation of cane sugar, glucosides, inulin, polypeptids, etc., one of the great advantages derived by the cell from its condensation activities is maintenance of reasonable levels of osmotic concentration within its body.

In the drying seed, of course, osmotic activity of the soluble materials is limited by the deficiency of water. Since osmotic pressure is really the pressure of water flowing into a region because of a difference in the forces which govern water balance, there seems to be little possibility of the development of strong pressures in the cells of embryo and endosperm tissues during the later stages of development of these structures; for there is too little water present to permit the soluble materials to create the typical unbalanced water pressure. The forces of movement may be unbalanced, but if the water is held tightly in the colloidal materials, it may not flow rapidly, just as in soils the film water movement becomes negligible when it reaches a certain thinness of film.

It is a well known fact that air dry substances of seeds have a very powerful attraction for water. The attraction between the water and solids of the seeds is mutual, and in part the result of molecular and ionic surface forces. This mutual attraction when measured is found to be at least hundreds of atmospheres. And oven dried seeds and water attract each other with forces of thousands of atmospheres. The causes of the attraction are never simple, but involve colloidal forces, surface chemistry, hydration, capillarity, etc., all of which may be summed up as imbibition forces. Only a small amount of the attraction is caused by the presence of soluble substances deposited

[2] While this paper was in press, a paper was published by E. PHILLIS and T. G. MASON on the polar distribution of sugar in the foliage leaf, in which movement of sucrose against its gradient of diffusion from mesophyll cells to phloem of the fine veins is apparently demonstrated (Memoir no. 4, Cotton Research Station. Trinidad. 1933).

in the air dry cells of the embryo and endosperm. It must not be forgotten, however, that if any important quantity of soluble compounds were to be held in solution in the small amount of water held by the air dry seed substance, the osmotic concentration might be fairly high, even when the osmotic pressure is nil.

Some years ago attention was drawn to the water-soluble substances of air dry seeds in a way that challenged the interest of the senior writer. BOUYOUCOS and MCCOOL (1), who were interested in the "bound" water which organic matter holds against the crystallizing forces of freezing, found that from one-fourth to three-fourths of the water imbibed by seeds could be held in unfrozen condition in the presence of freezing temperatures. Such protein-high seeds as alfalfa would imbibe as much as 127 per cent of their own weight of water, and almost 75 per cent of this imbibed water remained unfrozen at -3° C.

In order to determine the quantity of soluble material in the seeds, they ground them to a fine powder, treated definite quantities of the seed powder with definite quantities of water, and after a definite lapse of time determined the freezing point depression of the mixture by means of the Beckmann apparatus. Thus 10 gm. of powdered spring wheat, plus 20 cc. of water, after standing 40 minutes for solution of material, gave a freezing point depression of -0.280° C.; alfalfa seed powder gave a depression of -0.610° C.; and speckled wax beans a depression of -1.180° C. These depressions indicated osmotic concentrations (with this degree of dilution) of 3.375 atm. in the spring wheat powder, of 7.349 atm. in alfalfa, and of 13.336 atm. in speckled wax beans. As these depressions were obtained after adding relatively large volumes of water to the dry materials, these investigators argue for very high osmotic concentrations of soluble materials in dry seeds. They attribute to this great concentration of readily soluble materials in the cells of dry seeds their enormous power to abstract water from even relatively dry soils.

Without attempting any quantitative studies of their extracts, BOUYOUCOS and MCCOOL offered a suggestion as to the nature of the soluble materials which might be responsible for the high osmotic concentrations indicated by their freezing point determinations. It was this suggestion that challenged the interest of the senior writer

many years before an opportunity presented itself to test their conclusions. In the first place, they assumed that there are no soluble sugars in dry seeds, since the condensation processes lay them all down as starch, cellulose, and other insoluble hexosan and pentosan residues. It was argued, also, that there are not sufficient free salts in seeds to account for the observed depression of the freezing point in seed powders, and that therefore the soluble proteins, such as seed albumens, are mainly responsible for the highly concentrated solutions. To quote their concluding statement: "All evidences, therefore, point to the proteins as the main class of constituents in seeds which produced such high depressions in the freezing point when dry seeds in the powdered form were mixed with water."

One cannot take this statement literally, of course, as there was really no evidence submitted with regard to any of the constituents, only arguments based on common beliefs in regard to sugars and salts. In view of the high molecular weight of the simplest of soluble proteins, it would seem necessary to "dissolve" something like 25 gm. of protein in 1 gm. of water to produce a freezing point depression of $-1.18°$ C.

In this paper the writers present some evidence obtained over a period of years, the results of studies of seed extracts with special reference to the quantities of salts, sugars, and proteins which go into solution from powdered seeds. When the work was begun, some years ago, it was not realized that the exact nature of a seed extract would be so largely influenced by the manner in which it is made. It was believed that very cold (near freezing) temperatures would prevent serious autolytic changes in the carbohydrates and proteins. Pure solutions of sugars hold up remarkably well for long periods of time at temperatures of $33°$–$34°$ F., as will be mentioned later. But it became evident, as the work proceeded, that unless precautions are taken to prevent autolysis there is enough to change the quantities of soluble materials in the extracts. Our results, based upon extracts made at different times and places, and with unperceived differences in technique, are not always in such close agreement as one would wish. But they are recorded as a contribution to our knowledge of seed extracts and their physico-chemical behavior. The first studies were begun at West Virginia University in 1928.

The senior writer is greatly indebted to Dr. P. D. STRAUSBAUGH of the Department of Botany, and to Dr. R. B. DUSTMAN of the Department of Agricultural Chemistry, in whose excellently equipped laboratory and with whose helpful advice the first extracts were made and tested. Thanks are due also to the administrative officers of West Virginia University for many courtesies and privileges extended to facilitate the work. Repetitions and extensions of the work were made in the laboratories at the University of Chicago during the years since 1928.

Materials and methods

Two carbohydrate-high and two protein-high seeds were chosen for the investigation. Japanese buckwheat and amber sorghum represented the first group; and mammoth clover and the Arlington cow pea represented the second group. An abundant supply of these seeds was furnished by the Department of Agronomy from their stock supplies. The seeds were first ground coarsely in a feed mill, but the Merker mill was relied upon for the final stages of grinding. It was found possible to reduce the material with this mill, even the tough hulls of buckwheat seeds, to 100-mesh size. As the mammoth clover seed was found to offer special difficulties because of its colloidal properties when ground to this degree of fineness, later work was done with it ground only to 60-mesh size. The freezing point depressions were measured by standard Beckmann apparatus, using either a salt-ice mixture or ether bath for freezing.

Extracts were made either by extracting the seed substances with cold water (32°–34° F.) or with hot alcohol. The earlier work was done with cold water; but it was found that with this method the reducing sugars in the extract were always larger in quantity than the non-reducing ones. As this result was not in accord with the results usually obtained when alcoholic extractions are made, the alcoholic extraction method was used as a check upon the water extraction method. It was felt that this would throw some light upon the amount of autolysis that can still occur at low temperatures (diastatic action, etc.). It will be evident later, when the data are presented, that the cold water extracts were made under conditions that seem to permit a degree of enzymic decomposition.

In making the cold water extracts, 100 gm. of the ground seed material, thoroughly mixed, was placed in a 1000 cc. Erlenmeyer flask with 500 cc. of distilled water. These flasks were kept in a cold storage room with temperature just above freezing. The mash was shaken up twice daily from May 8 to May 13. At the close of the extraction period the clear extract was decanted on to a filter (Whatman no. 2 paper with platinum filter cones). The extracting material, handled at all times in the cold room, was washed repeatedly with 25 cc. portions of water (all carefully drained, decanted, and filtered), until at the close of the washings there was exactly a half-liter of extract from 100 gm. of material. The only departure from this procedure was used with mammoth clover seed extracts. These refused to filter because of their great viscosity. It was necessary, therefore, to resort to centrifuging to remove the solids from suspension in this case. This method is not considered as efficient as filtration in the case of strongly viscous solutions. The centrifuging was done in portions at about 1800 r.p.m. The washing and centrifuging of the extract was continued until there was 1 liter of extract from the original 100 gm. of seed powder.

The extracts were then analyzed for three constituents:

1. Total dry matter and total ash from an aliquot of each extract were determined by gravimetric means. The ashing was done in an electric muffle furnace in broad platinum dishes.

2. From other aliquots of the extracts, cleared of colloids by neutral lead acetate and deleaded with potassium oxalate, determinations were made of reducing and non-reducing sugars. In some of the determinations, gravimetric methods (copper reduction) were used, taking every precaution possible to obviate the usual sources of error in gravimetric procedures. In other cases, the SHAFFER-HARTMAN iodometric method was used. In particular, the reducing power of the alcoholic extracts was determined by the micro SHAFFER-HARTMAN sugar method (STILES ET AL. **6**). Inversion of the non-reducing sugars was carried out with 1 per cent HCl at the temperature of the boiling water bath for 2 hours. The difference between the reducing power after inversion and that before inversion is considered a measure of the non-reducing sugars. Mammoth clover seed extracts produce a gelatinous precipitate at the moment of addition of

the alkaline constituent of Fehling's solution. As this interfered with gravimetric determinations of reducing power in these extracts, the iodometric method was used instead of gravimetric determinations in this case. Careful tests made with known quantities of sugars, used separately and with the extract, showed that the method was sufficiently accurate with reference to the sugar recovered.

3. Total nitrogen of the extracts was determined by drying an aliquot (duplicates) in a Kjeldahl flask on a water bath, after which the salicylic modification to preserve nitrate nitrogen was used, with Hg (5) as catalyst, and with cochineal indicator in the final titration. The results of many of the measurements made are recorded in the following section. It is not practicable to attempt to present more than a fraction of our data, which are limited to the needs of the discussion.

Results

Freezing point depressions

The freezing point depression obtained from freezing a mixture of seed substance in powdered form with water will depend upon the degree of dilution employed. In table I are shown some of the depressions observed with the four seeds used, and some of the variations which were noted when the amount of water was varied in the case of the Arlington cow pea meal, and with the viscous suspensions of mammoth clover seed powder.

In the case of the Arlington cow pea, the electrical conductivity of the various dilutions was measured. The results, expressed in reciprocal ohms (mhos), are given in table II.

The changes in osmotic concentration (freezing point depression) and conductivity by dilution of the seed substance with water are shown graphically in figure 1. As is to be expected, the conductivity and freezing point depressions decrease with dilution, but the relations are not linear. The more dilute solutions have relatively greater conductivity and greater depression of freezing points than the more concentrated ones. This is probably partly to be explained by a more complete ionization of the electrolytes in the dilute solutions, or to the greater degree of "activity" of the ions in the greater dilutions.

The data of table I confirm in a general way the findings of Bouyoucos and McCool, that ground seeds mixed with water produce solutions of concentration sufficient to cause marked depression of

TABLE I

FREEZING POINT DEPRESSIONS OBTAINED WITH MIXTURES OF SEED SUBSTANCE AND WATER

Variety		Weight of seed (gm.)	Water (cc.)	Thermometer zero (°)	Freezing point* (observed) (°)	Depression* (°C.)	Osmotic pressure (atm.)
Black amber sorghum	I...	10	40	5.130	5.011	0.119	1.435
	II..	10	40	5.130	5.015	0.115	1.387
Japanese buck wheat	I...	10	40	5.130	5.010	0.120	1.447
	II..	10	40	5.130	5.010	0.120	1.447
Arlington cow pea	I...	10	20	5.130	4.454	0.676	7.866
	II..	10	20	5.130	4.505	0.625	7.529
	I...	10	40	5.130	4.818	0.312	3.761
	II..	10	40	5.130	4.820	0.310	3.737
	I...	10	60	5.130	4.935	0.195	2.351
	II..	10	60	5.130	4.940	0.190	2.291
	I...	10	80	5.130	4.970	0.160	1.930
Mammoth clover	I...	10	20†	5.910	5.643	0.267	3.221
	II..	10	20†	5.910	5.644	0.266	3.209
	I...	10	80	5.130	4.954	0.176	2.134
	II..	10	80	5.130	4.956	0.174	2.098

* Figures in these columns are averages of three or four determinations differing usually not much more than 0.002° C.

† Mammoth clover seed 10:20 was so pasty that good stirring was not possible. The depression values are believed not to be very accurate at this concentration, possibly involving water only in the interface between the thermometer and the seed mass. At this concentration the depression should probably be much greater, as indicated by the cow pea data.

TABLE II

Seed substance (gm.)	Water (cc.)	Conductivity (mhos)
10.....	20	0.002974
10.....	40	0.001676
10.....	60	0.001174
10.....	80	0.000950

the freezing point. In none of our measurements, however, did we find as high depressions as were reported by these investigators in certain cases. It may be remarked that it is very easy to exaggerate

the depression of the freezing point by having the bath too much colder than the material being tested. The bath must be kept only slightly below the material in temperature, regardless of the greater time it takes for a measurement, or the heat of freezing will not register its full amount. We took extreme care in our work not to make the energy gradient too great. But differences in preparation, and

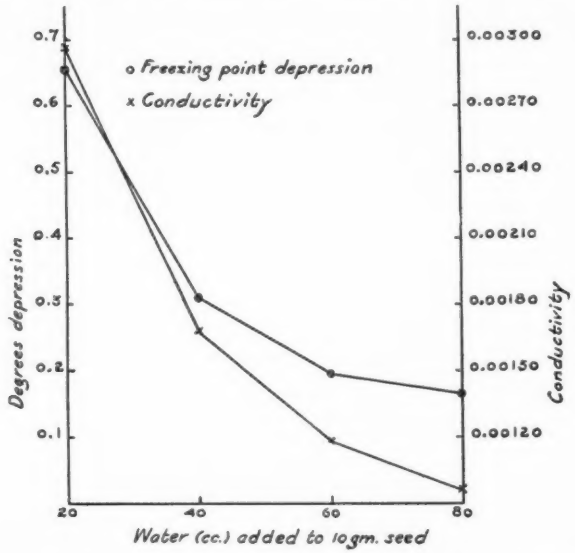


FIG. 1.—Changes in freezing point depression and conductivity with dilution

differences in material used, are probably more responsible for our somewhat lower values.

An effort was made to determine whether the time elapsing between the addition of the water to the seeds and the determination of the freezing point would make any notable changes in the values obtained. Two series of tests were made with the Arlington cow pea (10:40) which show that the soluble constituents go into solution rather rapidly, or at least become able to affect freezing point depressions, and that the concentration does not increase materially on standing, at least during the first few hours. On long standing, of

course, autolytic decompositions would make a difference. But whether a measurement is made after a few minutes or after a couple of hours makes only a rather small difference in the results.

The data for two of these samples are summarized briefly in table III. Sample I shows a slight tendency to change with time, but sample II remained practically constant for five hours. Slight inequalities in the samples chosen for the tests or imperfect mixing might rather easily produce results as irregular as they appear in

TABLE III

	Sample I		Sample II	
	Thermometer zero	Freezing point	Thermometer zero	Freezing point
Immediate	5.130	4.663	5.130	4.505
1 hour	5.130	4.460	5.130	4.505
3 hour	5.130	4.460	5.130	4.502
5 hour	5.130	4.430	5.130	4.505

sample I. We are inclined to think that there is prompt solution of such materials as are soluble, and that only after some hours of standing do the diastatic and other autolytic fermentations disturb the relations seriously.

Total solids

The analyses for total solids were run in duplicate on two different sets of samples, prepared at different times and places, and probably under unappreciated or unrecognized differences in handling. About the same technique was used in preparing the samples, except that the temperatures could not be made identical in the two cases, a very important variation, even if the differences were small. This could not be avoided. Another important difference was the mesh size of one of the samples. In the case of mammoth clover, the first extract was made from 100-mesh seed powder; the second was ground only to 60-mesh because the first one was too difficult to handle, too viscous, too hard to clear. While these differences in method make considerable difference in results, they cannot be said to vitiate the general conclusions which may be drawn from the investigation. It is

obvious from the large discrepancy in the mammoth clover data (table IV) that the 100-mesh material could not be cleared as completely by centrifuging as was the 60-mesh sample. That this is not the whole explanation, however, is evident from the difference between mammoth clover I_1 and I_2, table IV. Sample I_2 was taken from a solution which had passed through the Whatman no. 2 filter, while sample I_1 was pipetted from the perfectly clear limpid supernatant liquid which had settled completely, but had refused to filter. While sample I_1 might have contained, and probably did contain, some undissolved colloidal aggregates, sample I_2 could hardly have done so. Evidently the fineness of grinding here increased the solubility in sample I_2 about 6 per cent above samples III_1 and III_2, almost a 40 per cent increase in solubility.

In the case of the other seeds, the duplicate determinations from individual runs check closely enough, but the different runs differ from unknown causes. It seems best to record the data and average the values of the determinations (table IV). The figures indicate at least the general order of solubility, and the data throw a certain amount of light on the problems of solubility of seed substance when in a fine state of subdivision.

The data in table IV indicate that a very considerable portion of the substance of a seed may go into solution when it is extracted for a period of five or six days with water. It should be stated, also, that there were never the slightest indications of bacterial or mold growth in any of the flasks during preparation of the extracts. A check on the stability of carbohydrates away from their hydrolytic and respiratory enzymes was obtained from unsterilized standard sugar solutions that were placed in the cold chambers in February and kept until June. Polarized at the start of the tests, they were polarized again in June, and showed no measurable change in sugar concentration during four months of storage at 32°–34° F. There is in our extracts strong evidence of the occurrence of autolytic changes with time. No doubt this should have been expected, since invertase activity is not prevented at zero, and many of the enzymes may act slowly at this temperature. The substances that cause freezing point depression go into solution quickly, and give as large depressions in a few minutes as they do in a few hours; but on long standing, autol-

ysis would certainly cause an increasing amount of carbohydrates to appear in the extracts. Proteins may also be affected, but the change would be in a direction to favor the BOUYOUCOS-MCCOOL suggestion rather than to discredit it.

TABLE IV

TOTAL SOLIDS OF SEED EXTRACTS IN PERCENTAGE OF ORIGINAL DRY WEIGHT

SEED EXTRACTED	ORIGINAL DRY WEIGHT (%)	
Black amber sorghum I_1	4.66	
I_2	4.64	
II_1	8.08	
II_2	8.02	
Average	6.36	
Japanese buckwheat I_1	9.86	
I_2	9.94	
II_1	11.74	
II_2	11.53	
Average	10.77	
Arlington cow pea I_1	16.79	
I_2	16.93	
II_1	18.98	
II_2	19.01	
Average	17.93	
Mammoth clover I_1	24.50	100-mesh
I_2	21.75	
II_1	24.49	
II_2	24.48	
III_1	15.91	60-mesh
III_2	15.33	
Average (all)	21.08	

TOTAL SOLUBLE CARBOHYDRATES

Although analyses of seeds usually show the presence of some carbohydrates in soluble form, it is usual to find the non-reducing sugars more abundant than the reducing sugars.

Several years ago MACGILLIVRAY (**4**) reported analyses of tomato seeds from fresh tomato pulp. He found from 3.23 to 3.70 per cent of free reducing sugars (dry weight basis), calculating them as glucose. These seeds had not been allowed to dry after removing them from the pulp, nor were they washed free of sugar before preserving in alcohol, a circumstance that might favor the presence of a larger amount of free reducing sugar than is usual in seeds when thoroughly washed and dried. KRAYBILL (**2**) reports that sucrose occurs in wheat,

varying in quantity in the different varieties, the percentages being as follows: Shepherd 1.61, Gladden 1.53, Fulhard 1.52, Nittany 1.65, Purkhoff 1.55, Kawvale 1.53, and Fultz 1.25 per cent. He also states that almonds, castor beans, and cucumber seeds contain from 1 to 2 per cent sucrose; and that he has isolated sucrose pure from soy beans, which contain a considerable amount of this sugar. It is obvious that one cannot assume the absence of sugars in dry seeds.

Our extracts, which were made with prolonged contact with cold water, indicated, as has been stated, that the reducing sugars were more abundant than the non-reducing types. This is frankly ac-

TABLE V

TOTAL, REDUCING, AND NON-REDUCING SUGARS IN COLD WATER SEED EXTRACTS (DRY WEIGHT BASIS)

SEED EXTRACTED		REDUCING SUGAR (%)	NON-REDUCING SUGAR (%)	TOTAL SOLUBLE SUGAR (%)
Black amber sorghum	I	1.58	1.31	2.89
	II	1.57	1.36	2.93
Japanese buckwheat	I	2.18	0.12	2.30
	II	2.08	0.16	2.24
Arlington cow pea	I	5.32	0.75	6.07
	II	5.29	0.84	6.13
Mammoth clover	I	1.91	1.48	3.39
	II	1.84	1.49	3.33

cepted as evidence that, in spite of the near zero temperatures, some autolytic changes occurred that increased the quantities of sugar determined. That we might not overestimate the rôle of soluble carbohydrates in the depression of freezing points of seed extracts, some hot alcoholic extracts were made so that comparisons might be made with the water extracts. The results are presented in tables V and VI. Table V gives the data for the cold water extracts.

The smaller total quantities of sugars were found in the carbohydrate-high seeds, such as sorghum and buckwheat; and somewhat larger amounts were found in the protein-high seeds, mammoth clover, and particularly in the Arlington cow pea. It was the unusual amount of reducing sugars in the cow pea, and the uniform predominance of the reducing sugars over the non-reducing, that led to the knowledge that autolysis was an important factor when seeds are

extracted for long periods in the cold. The hot alcoholic extractions yielded very low results for both reducing and non-reducing sugars, certainly not so high as many other seed analyses would lead one to expect. The results are given in table VI. These determinations were made by the iodometric method (micro SHAFFER-HARTMAN), with 1.5 gm. samples (100-mesh or 60-mesh) which were extracted with 80 per cent alcohol for three hours. The figures are in each case the average of four determinations. From these data it seems that sugars are either much less abundant in seed extracts than the data from cold water extractions would lead one to believe, or that they

TABLE VI

TOTAL, REDUCING, AND NON-REDUCING SUGARS IN HOT ALCOHOLIC SEED EXTRACTS (DRY WEIGHT BASIS)

SEED EXTRACTED	REDUCING SUGAR (%)	NON-REDUCING SUGAR (%)	TOTAL SOLUBLE SUGAR (%)
Black amber sorghum	0.08	0.17	0.25
Japanese buckwheat	0.07	0.07	0.14
Arlington cow pea	0.18	0.19	0.37
Mammoth clover	0.33	0.11	0.44

do not extract readily with hot alcohol. Probably autolysis has multiplied the sugars in solution in the case of the data in the last column of table V.

Nevertheless, from all of the facts presented it appears that there are sugars in dry seeds; and that while the quantities may be small, rarely exceeding 2 per cent, and frequently falling below that amount, yet they are not negligible in the problem of freezing point depressions of seed extracts. Especially are they not negligible when the freezing point depressions are small.

TOTAL ASH

The dried aliquots used in obtaining total solids in solution were carefully ashed at low red heat to avoid serious volatilization of bases. The different extracts again showed some variations. Duplicates from a single extract checked closely; but evidently the details of preparation would have to be very closely standardized to secure

concordant results from different extracts. The data on total ash found in the various extracts are presented in table VII. They give a fair measure of the total ash elements which occur in such seed extracts.

These results (table VII) show the presence of from approximately 1 per cent to nearly 3 per cent of ash in the form of oxides of the

TABLE VII

TOTAL ASH DETERMINATIONS ON SEED EXTRACTS
(DRY WEIGHT BASIS)

VARIETY OF SEED		ORIGINAL SAMPLE (%)
Black amber sorghum	I_1	0.956
	I_2	0.952
	II_1	1.34
	II_2	1.35
Average		1.15
Japanese buckwheat	I_1	1.286
	I_2	1.282
	II_1	1.280
	II_2	1.270
Average		1.28
Arlington cow pea	I_1	2.82
	I_2	2.91
	II_1	2.58
	II_2	2.55
Average		2.75
Mammoth clover	I_1	2.686
	I_2	2.684
	II_1	1.58
	II_2	1.55
Average		2.12

basic elements. While the results are somewhat variable, they establish a sound basis on which to estimate the ash influence in the freezing point behavior of finely ground seeds.

TOTAL NITROGEN OF EXTRACTS

Total nitrogen was determined by methods already stated, and the results were used in calculating the amount of protein in the extracts. No attempts were made to fraction the nitrogen, and total $N \times 6.25$ is accepted as a conventional approximation of the quantity of protein dissolved in the extracts. In table VIII are shown the total nitrogen determinations, and the corresponding calculated percentages of protein present.

It is seen by examination of the data that the results with the protein-high seeds are not at all comparable in the two sets of extracts. In one instance the extract of mammoth clover seed contains 50 per cent more protein in solution than the other extract. Here again it is the extract from 100-mesh material that runs higher than the determinations from 60-mesh powder. The differences in the cow pea

TABLE VIII

TOTAL NITROGEN DETERMINATIONS ON SEED EXTRACTS
(DRY WEIGHT BASIS)

VARIETY OF SEED		SOLUBLE NITROGEN IN EXTRACTS FROM ORIGINAL SAMPLE (%)	SOLUBLE PROTEIN IN ORIGINAL SAMPLE (%)
Black amber sorghum	I_1	0.032	0.20
	I_2	0.037	0.231
	II_1	0.0256	0.160
	II_2	0.0248	0.155
Average		0.299	0.187
Japanese buckwheat	I_1	0.258	1.613
	I_2	0.262	1.638
	II_1	0.267	1.669
	II_2	0.266	1.663
Average		0.263	1.645
Arlington cow pea	I_1	0.96	6.00
	I_2	0.96	6.00
	II_1	0.329	2.056
	II_2	0.329	2.056
Average		0.645	4.028
Mammoth clover	I_1	0.65	4.063
	I_2	0.67	4.188
	II_1	1.0184	6.365
	II_2	1.0074	6.296
Average		0.836	5.228

data are not easily explained. The extracts were made in the same manner, and so far as known, all of the procedures were essentially the same; but one set runs nearly three times as much soluble protein as the other. There is fair agreement in the case of Japanese buckwheat, and both sets of analyses agree in the small amount of proteins occurring in the extracts of black amber sorghum. It is not so much absolute quantities and close agreements that are needed for this problem, however, as general notions of the amount of proteins that are soluble in such seed powders. The data give such approximations, regardless of the irregularities which they show.

We have now given such approximations for carbohydrates, ash elements, and proteins. When the totals of these are compared with the total solids of the extracts, they by no means account for all of the soluble materials. For example, in the extract of the Arlington cow pea, one of these analyses shows 18.98 per cent total solids; of this total, 16.40 per cent is organic and 2.58 per cent inorganic. The total sugars account for 6.07 per cent of the organic material, and the dissolved proteins account for 6.0 per cent. The remainder is not determined. We are not trying to make a complete determination of all of the soluble material, but merely to establish the general situation as to the main soluble constituents, salts, sugars, and proteins. With the facts at hand it is possible to consider intelligently the problems of freezing point depressions as observed in such seed powders when frozen with definite quantities of water.

Discussion

The data presented in the preceding section of this paper we believe indicate clearly that there are appreciable amounts of both ash elements and carbohydrates in the substance of seeds, and that these substances are capable of going into solution quickly when the finely divided substance is immersed in water. The matter that interests us mainly, however, is whether there is a large enough quantity of these materials present to account for the depression of freezing points that are observed in such extracts or mixtures of seed powder and water when frozen, and to produce the osmotic concentrations which these depressions indicate (1.5–8.0 atm.), as shown in table I.

It is not necessary to enter upon any lengthy discussion of this subject; an example will serve to make clear the significance of our results. Take, for example, the case of the Arlington cow pea, in which case a 10:40 mixture of seed substance and water gave an observed depression of freezing point of 0.312° C., corresponding to an osmotic concentration of 3.761 atm. (table I). The data of table VII show that the average amount of ash for four samples of this seed was 2.75 per cent; and from two analyses in table VIII, the sugars were found to be 6 per cent. As has already been suggested, these sugar figures may be too high; but how much too high must be left to others to determine. The evidence from hot alcoholic extractions

can hardly be applied, since all freezing point depressions are measured in cold water mixtures with ample time for solution. At any rate, the treatment of this example illustrates the reasoning to be applied to any such case, whether the sugar data in this particular instance are reliable or not.

What do these figures of ash and sugar concentration mean in terms of freezing point depression? In the first place, if there are 2.75 per cent of ash elements present, the 10 gm. sample of powder will contain 0.275 gm. of ash. Furthermore, since the mixture was a 10:40 one, there is but one twenty-fifth of a liter of water present. The ash is present, then, at the rate of 25×0.275, or 6.875 gm. per liter. If one were certain of the average weight of the ions present, it would not be difficult to determine about how much depression of freezing point should result from the action of the dissolved salts. We do not know the average weight of the ions, nor do we know the extent to which they are ionized in the seed substance when immersed. If the ash ions weighed on the average about as much as Ca, K, Mg, etc., it would require only 35 or 40 gram-ions per liter to account for a depression of $-1.86°$ C., and a corresponding osmotic concentration of 22.4 atm., supposing complete ionization. Under such circumstances, 6.875 gram-ions per liter would be equivalent to about one-sixth molar solution, and should cause a freezing point depression corresponding to an osmotic concentration of about 3.7 atm. This is as much as the entire depression observed in the case of the Arlington cow pea. But, it may be argued that these basic ions are not paired with simple inorganic anions, but accompany complex organic anions of protoplasm and other structural ingredients which are not osmotically active. In that case the weight of ash is greater than the actual weight of inorganic ions in the tissues; for in the ash they are all oxidized, and we weigh the added oxygen as a part of the ash. Of course there are some simple inorganic anions in the tissues, chloride, sulphate, phosphate, nitrate, etc., but still it is probable that ash is considerably heavier than the real weight of inorganic ions in the tissue. It would be more than generous, however, to allow as much as 50 per cent of the weight of ash for the increase in oxygen. If we estimate that the actual weight of the ions is only half of the observed weight, then we would have to halve the

effects of the salts, and instead of crediting them with the entire osmotic action (in table I there is only 3.761 atm. for which to account), we would have to attribute possibly 1.85 to 2 atm. to the salts alone. This is discounting them very liberally.

The carbohydrate situation is equally interesting. In table III it is shown that there is 5.3 per cent of reducing sugar and 0.8 per cent of non-reducing sugar in the cow pea extracts, or about 6.0 per cent total sugar. This means that a 10-gm. sample would contain 0.6 gm. of sugar, or at the rate of 15 gm. per liter in a 10:40 mixture. Such a solution, if composed of hexose reducing sugars only, would possess the osmotic concentration of a twelfth-molar solution, equivalent to 1.8 atmospheres. It is of course actually less, since some of the sugar is of non-reducing type, with heavier molecules. Moreover, it is entirely possible, or even probable, that these figures are too high. But even a 2 per cent soluble sugar content would bring about a depression of freezing point equivalent to a concentration of from 0.3 to 0.6 atm., depending on whether it were sucrose or dextrose.

The figures obtained from hot alcoholic extracts would give almost no influence to sugars. But since our freezing point tests were all made in aqueous mixtures, we are using the figures obtained from aqueous extracts for our arguments.

It must be obvious from the data that almost the entire freezing point depression caused by the Arlington cow pea meal when frozen in water is accounted for by our data on salts and carbohydrates. It would seem fair to make the claim that in a general way these two factors are most largely responsible for the freezing point depression. There are, of course, other soluble substances present which have not been determined, and which must play their part.

Finally, the proteins should be mentioned briefly. The data in table VIII indicate roughly the presence of from 0.15 to 6.3 per cent of soluble protein in the various extracts as made. If the nitrogen really represents proteins, and not amides and amino acids, their effects on freezing points would have to be utterly negligible. Even with 6.3 per cent protein, a 10 gm. sample would contain but 0.63 gm., or at the rate of 15.75 gm. per liter of water in a 10:40 mixture. This amount would represent about one two-thousandth of the

gram-molecular weight of a light weight protein; and this concentration should theoretically produce a freezing point depression corresponding to an osmotic concentration of 0.011 atm. (about 0.001° C. of depression).

It is concluded, therefore, that the depression of freezing points, and the osmotic concentrations shown by finely powdered seed substance when frozen with water, are caused by the salts and sugars, which are present in sufficient quantity to account satisfactorily for the observed phenomena. There seems to be no reason for ascribing to proteins any special rôle in connection with the osmotic action of seed substances. They are undoubtedly as important in connection with imbibition phenomena as any other colloidal constituents of the seeds.

Summary

1. Freezing point depression determinations were made on mixtures of finely powdered seeds and water. The four varieties of seeds used were black amber sorghum, Japanese buckwheat, Arlington cow pea, and mammoth clover. These determinations, repeated many times, confirm in a general way the measurements of Bouyoucos and McCool, but with lower values.

2. Some conductivity measurements were made to throw light upon the effects of dilution on the electrolytic constituents of the seed extracts.

3. Analyses of cold water extracts of the seed powders are presented, covering total solids, total soluble carbohydrates (reducing and non-reducing sugars), total ash, and total nitrogen (proteins).

4. These analyses, coupled with analyses of alcoholic extracts, indicate that autolysis occurs even at temperatures but slightly above freezing.

5. Rough calculations have been made, using the values obtained in the analyses for ash, sugars, and proteins, to determine the approximate influence of each of these substances in producing freezing point depressions and osmotic action.

6. From these calculations it is concluded that almost all of the freezing point depression can be accounted for by the quantities of salts and sugars present.

7. There are other unknown and undetermined soluble constituents present in the extracts, whose action must also play a part in freezing point depression and osmotic action.

8. The proteins are practically negligible as a factor in the freezing point behavior of seed extracts.

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DEVELOPMENTAL MORPHOLOGY OF ALLIUM CEPA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 448

CHARLES ANDREW HOFFMAN

(WITH TWENTY-NINE FIGURES)

Introduction

Of all the Liliaceae, the onion, *Allium cepa* L., is of greatest economic importance. Its distribution is world-wide and its popularity as an article of food is almost as extensive. SACHS (**10**), in a well illustrated article, discusses the microchemistry of the seed and seedling, together with the anatomy and germination of the seedling. SIDERIS (**11**) notes that onion roots emerge in two sets, one group for the early life of the seedling and another for bulb formation. The first roots die as the second group becomes functional. JONES and BOSWELL (**5**) found that the primordia of the flower axis in bulbs which were formed the previous season are differentiated during March. POESE (**8**) notes that the vascular bundles in all the Liliaceae he investigated fluctuate between a collateral and a concentric type, the latter appearing most commonly in the rhizome and at the nodes. MANGIN (**6**) states that the meristem giving rise to adventitious roots is absent in all monocotyledons without these roots.

MATERIAL AND METHODS.—In the fall of 1930 and in the following summer, material was grown from pedigreed seed of the Yellow Globe Danvers onion. From this material the various stages to be studied were selected. All the material, except the primary roots which were killed in Flemming's medium fluid, was killed in a mixture of four parts absolute alcohol and one part glacial acetic acid, passed through five mixtures of ethyl and butyl alcohol, and finally imbedded in paraffin as described by ZIRKLE (**13**). The primary roots were cut 6μ thick and the other material from 8 to 15μ thick.

Embryo

The mature embryo is a curved cylinder about 0.4 mm. in diameter and 6 mm. long, of almost uniform width, somewhat pointed

at the hypocotyledonary end and rounded at the opposite or haustorial end. It is almost completely surrounded by an endosperm whose cells contain fat (10), and whose walls are much thickened with a hemicellulose which on hydrolysis yields mannose (2). The haustorial end may be merely curved at right angles to the remainder of the embryo or may form more than a complete circle. A 270° arc is the average. About one-tenth of the length of the embryo is hypocotyl; the rest is cotyledon. Proximal to the tip of the hypocotyl, a distance twice the diameter of the embryo, is a slit in the cotyledon or first leaf through which the second leaf grows. This slit and the cavity inside it are usually filled with a waxy or gummy substance. The cavity thus formed by the hollow base of the cotyledon surrounds the primordium of the second leaf, which usually is tongue-shaped, with a length about twice its thickness. The slit, according to SACHS (10), occurs with equal frequency on the concave and on the convex sides of the embryo.

All of the cells of the embryo are very thin walled. The five regions of the embryo as seen in longisection and described by SACHS are clearly distinguishable. They are: (1) the peripheral layer, which a week after planting has formed the epidermis of the cotyledon together with its stomata and guard cells; (2) the parenchyma of hypocotyl and of cotyledon which, even in the embryo, has many intercellular spaces; (3) the single procambial strand running from the promeristem of the root tip to the stem region and up through the cotyledon to the peripheral layer of the haustorial portion. The cells of this procambial strand and of the parenchyma are in long rows. In some embryos the procambial strand contains spiral elements which are first differentiated above the region of the stem, a distance two or three times the diameter of the embryo; (4) the promeristem of the root and of the stem tip; (5) the root cap.

Germination

SACHS' account of the changes which occur in the form of the embryo and in the content of the cells during germination was confirmed. Development of the embryo begins with elongation of the lower and middle regions of the cotyledon. Within 24 hours the hypocotyl is pushed through the seed coat and soon thereafter most

of the cotyledon also is pushed out. The tip of the cotyledon, however, remains imbedded in the endosperm of the seed from which it absorbs food. When 4–6 mm. of the embryo is outside the seed coat, a bending in the cotyledon occurs above the slit so that the hypocotyl is directed downward. By the end of a week the root has grown downward 4–6 cm., while the short stem region has remained at about the same level as that occupied by the seed at the time of planting, and the cotyledon has attained a length of 8–12 mm. In the cotyledon, midway between the slit and the attached seed, a region has become sharply bent double so as to present its convex side to the soil surface above it. This is the soil-penetrating organ or "knee," which is carried upward by elongation of the part of the cotyledon on either side of it.

Apparently there is no localized meristematic region in any part of the cotyledon, cell divisions occurring throughout its entire length, although the cells at each end reach their full size later than the others. After becoming vacuolate and six to eight times their embryonic length, the cells of the cotyledon divide not more than two or three times and elongate slightly. During the first five days no mitotic figures are found in the cotyledon, the growth being due to cell enlargement alone. A few days later figures become abundant but two weeks later are absent. Mitoses occur in all tissues but last to begin is the region near the seed. After eight or ten days, elongation of the seed limb of the cotyledon ceases, while the stem limb, especially near the stem region, continues to elongate slightly. This causes the stem limb to be curved and the seed limb to be stretched across the curve like a bowstring. By this time the haustorium has absorbed so much of the endosperm that it lies loosely in it, and, under the growth tension of the cotyledon, the haustorium is pulled out of the seed and germination is completed. At this time the cells in the concave side of the knee grow until finally the seed limb of the cotyledon also points upward, although a bend always marks the location of the knee. The haustorium, once out of the seed, immediately withers but the rest of the cotyledon remains photosynthetic.

By the time germination is completed the primary root has attained almost its full length of 10–12 cm. It does not branch, and all subsequent roots originate adventitiously from the stem. Just

above the region of root hairs at the top of the hypocotyl the first two or three adventitious roots push out through the outer cortex of the lower stem. This action seems to be both lysigenous and schizogenous. These roots originate from the parenchyma of the inner cortex, which becomes meristematic (fig. 11). The first of these roots arises from the cortical sector below the slit. The next appears usually more than 90° to the right or left of the first, and if there be a third, it is developed in the region diametrically opposite the second. Subsequent adventitious roots originate as does the first. There is no regularity in the order or place of their appearance. Each successive root is usually less than half its own diameter above the previous one. A plant five months old may have had well over 100 root primordia and roots.

Primary root

The primary root is an exarch radial protostele, usually diarch but sometimes triarch. When both xylem and phloem are mature there is no parenchyma in the stele except the uniseriate pericycle. The central metaxylem consists of one to five, usually two, larger elements which mature into scalariform vessels (fig. 9). Casparian strips are apparent in a seedling six days old, but never become very thick. Cortex and epidermis are parenchymatous.

Development of the root axis is like the "type 2" of JANCZEWSKI (4) as modified by TREUB (12). The meristem consists of two regions, the one giving rise to the plerome and the other to the periblem, epidermis, and root cap (fig. 15).

As seen in longisection the stele appears to arise from two, three, or four cells at its distal end. From the derivatives of these a pericycle is first differentiated, and then one of the centrally located cells enlarges and thereafter does not divide so often as those surrounding it but grows in all dimensions, especially longitudinally. From a row of such cells the large central vessel is formed. One or two periclinal divisions of the surrounding cells bring the stele to its diameter of five to ten, usually seven, cells. Subsequent cell divisions take place transversely and thus longitudinal growth continues. The cells of the pericycle are slightly shorter than those of the endodermis and neighboring cortical cells, but are more easily distin-

guished because they are immediately outside the very slender procambial cells.

Distal to the stelar histogen is a meristematic region three to six cells in diameter and two to four cells deep. From the derivatives of the peripheral cells of the upper layer or layers of this region the cortical cells are differentiated. From the derivatives of a lower layer of the same region is differentiated the single layer of epidermal cells which undergo no further periclinal divisions but divide only transversely and anticlinally. From the lowest layers of this meristematic region the root cap is differentiated. The cortex, epidermis, and root cap are first distinguishable as distinct regions at about equal distances from the center of the common histogen from which they are derived. One or two periclinal divisions of the cells of the region between the epidermis and stele give the cortex its mature width of from five to eight, usually six cells. The cortical cells have divided transversely more frequently than those of the stele and less frequently than those of the epidermis, and thus are intermediate in length between the two.

The root cap consists of several conical layers of cells of which the outer is oldest and largest. Near the apex of each cone the cells are larger, slightly more numerous, and elongated longitudinally, resulting in the wedge-shaped outline of the cap. At the center of the root cap, where there has been least distortion by growth, a longisection shows two to four somewhat irregular vertical rows of cells, each row still clearly traceable to a single cell of the histogen.

Vascular anatomy of cotyledon

Differentiation and maturation of xylem first begin above the slit and from this point proceed upward and downward at about equal rates. The protoxylem in the cotyledon, and other leaves as well, consists usually of spiral elements, although some annular thickenings may be present, especially in the tapering ends of the tracheids. In the cotyledon as seen in transection there is developed one protoxylem area and a band of metaxylem on each side of the younger part of this protoxylem. Eventually there is formed a stocky Y-shaped area of xylem with an accompanying area of phloem at the end of each upper arm of the Y (figs. 3–6). Sometimes

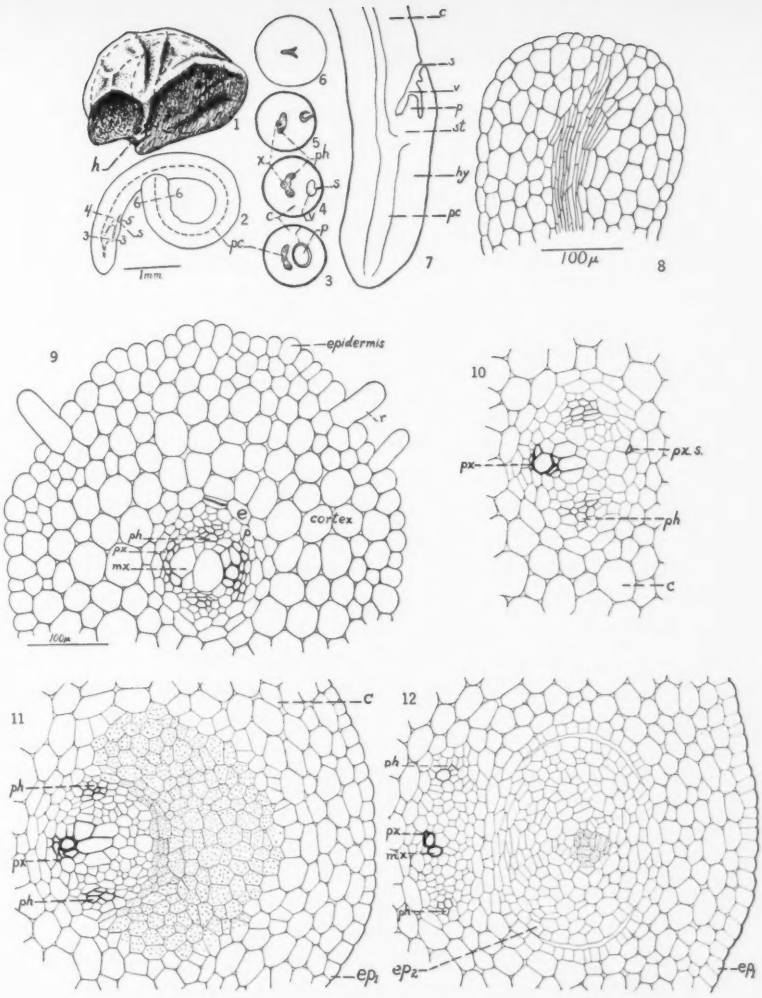
the xylem is separated into three or four groups by parenchyma, or the Y may be flattened like a short-stemmed T. Phloem matures first at the points farthest removed from the protoxylem.

In two seedlings, each with two growing points at the tip of the stem, there was only one cotyledon but two cavities, one for each growing point, and one vascular bundle in the cotyledon between the two cavities. The xylem of this bundle appeared in transection as an oblong band flanked on either side by a cavity with its growing point. The protoxylem was at the middle of one of the longer sides of the xylem area and a phloem group was at each of its ends. Thus in this case as well as normally development of the xylem was exarch. In one of the seedlings the primary root had four phloem groups and was probably tetrarch. The root of the other was not seen.

CHAUVEAUD (1) reports resorption of the protoxylem and its replacement *in situ* by metaxylem in the cotyledon of seedlings eight to ten days old. He figures ten transections but no longisections to show this. Actually no resorption occurs, but the protoxylem vessels become so stretched by the growth of the surrounding cells that their annular thickenings are so widely separated or their spiral elements so much stretched that the walls between thickenings collapse, and in transection the vessels are seen as only a very small patch of cellulose easily overlooked. This protoxylem becomes more difficult to find as the cotyledon becomes older. EAMES and MACDANIELS (3) report a similar situation in *Lobelia*. In longisection protoxylem is easily seen in all material less than four weeks old, at which age the second and third leaves have appeared and the cotyledon begins to wither from the tip downward toward its base. Development of the cotyledonary xylem is exarch throughout.

Anatomy of stem

In the germinating seedling all of the hypocotyl is rootlike in structure. Near the top of it the two protoxylem areas of the stele of the root appear rather abruptly, much separated from each other. The one extends into the cotyledon while the other ends at a distance equal to from one-third to one-half the diameter of the cotyledon beneath the primordium of the second leaf (fig. 7). The protoxylem of this latter strand is never extended up farther but ends blindly (fig. 10). Immediately above the level at which this protoxylem



FIGS. 1–12.—Fig. 1, seed showing position of embryo (drying of endosperm causes irregular wrinkling). Fig. 2, embryo dissected out of seed. Fig. 3, diagram of transection through embryo at level 3–3 of fig. 2. Figs. 4, 5, same, but at level 4–5, showing variation in structure at slit (*s*) and in outline of procambial strand (*pc*). Fig. 6, same, but at level 6–6. Fig. 7, diagram of longisection through portion of embryo. Fig. 8, longisection through tip of haustorium showing procambial cells extending to epidermis which is without a cuticle (*c*, cotyledon; *h*, hilum; *hy*, hypocotyl; *p*, leaf 2 primordium; *pc*, procambial strand; *ph*, position of future phloem; *s*, slit; *st*, stem; *v*, cavity in hollow base of cotyledon; *x*, position of future xylem). Fig. 9, transection through upper part of primary root of 6-day seedling. Fig. 10, transection 390μ above fig. 9, showing protoxylem under stem (*pxs*) consisting of single cell only. Fig. 11, transection 90μ above fig. 10, showing actively dividing cells of cortex (stippled) beginning formation of first adventitious roots. Fig. 12, transection 190μ above fig. 11, showing leaf 2 with first procambial strand (stippled) inside cotyledon (*c*, cortex; *e*, endodermis ep_1, epidermis of cotyledon; ep_2, same of second leaf; *mx*, metaxylem; *p*, pericycle; *ph*, phloem; *px*, protoxylem; *r*, root hair).

area ends, all of the xylem of the cotyledonary bundle and of the stem develops in one direction, away from the protoxylem of this cotyledonary bundle toward the center of the stem and even beyond it. At this level the four or five elements of the cotyledonary protoxylem are arranged side by side in a line which would pass perpendicularly to a radius of the stem extended through them. The metaxylem between this line of protoxylem and the point above the other protoxylem area appears in transection as a wedge-shaped area with two or three isolated metaxylem vessels at its point, but separated from the remainder of the metaxylem by parenchyma. Excluding the cotyledonary bundle, these isolated vessels are the first of the stem xylem to mature. Later at this level metaxylem matures on all sides of these isolated vessels, but at a slightly higher level, only on the side farthest from the cotyledonary protoxylem, that is, centrifugally. At the same time xylem matures also on each side of the metaxylem wedge as well as around its tip, but is separated from the wedge by a layer of parenchyma. Thus in transection there appears a thick U of xylem surrounding the wedge, except at its widest portion which is cotyledonary xylem. The base of this U is split off higher up to form the largest and central bundle of the second leaf. Part of the remaining arms form a portion of the two bundles of the second leaf, lateral to its largest bundle, and part continues upward to form the outer rather definite vascular layer of the stem (fig. 27). This outer layer, however, is formed largely from the steles of the adventitious roots.

The point at which parenchyma is first apparent in the upper hypocotyledonary xylem is arbitrarily called the bottom of the stem. In a seedling ten days old the stem is less than one-half as long as the diameter of the seedling and contains no matured xylem except the cotyledonary bundle. About ten more days are required for the first xylem to mature through this short length of stem up as far as the base of the second leaf. The lower part of this xylem consists of reticulate cells not more than three times as long as wide. The xylem slightly farther up is scalariform, while in the leaf the protoxylem is again of long spiral vessels which mature centrifugally.

In the center of the stem the vascular arrangement is determined largely by the manner in which the leaves develop. Each successive

leaf matures as an upright hollow cylinder, one side of which grows much more rapidly than the other, to form the blade, while the cylinder itself is the sheath surrounding the younger leaves and the apical meristem (figs. 24, 25). Because of the cylindrical sheath, therefore, the vertical vascular bundles of the leaf are arranged in a circle at its base. The largest bundle is near the center of the side forming the blade. The largest bundle of leaf 2 is farthest from the cotyledonary bundle and that of leaf 3 is near the cotyledonary bundle but separated from it by the sheath of leaf 2. In the stem the bases of the larger bundles of each leaf are connected with a horizontal U- or C-shaped ring of vascular tissue. Successively higher rings have vertical connections with each other, and thus a central parenchymatous area, the pith, is surrounded by a cylindrical network of vascular bundles. Lateral branchings extend from this central vascular cylinder toward the periphery of the stem, but are branched extensively both horizontally and vertically before they are anastomosed with the vascular layer limiting the stele. Over half of the stele is parenchymatous.

Leaf traces are never connected directly with the vascular tissue of the adventitious roots. The traces of larger bundles of the leaf are connected directly with the perimedullary vascular cylinder, while those of the smaller bundles are connected with the vascular tissue of the stem nearer its periphery. At the periphery of the stem the vascular tissue of the roots, with that of the outer stem, is spread out to form an almost continuous layer of intimately connected vascular bands outlining the stele of the stem. Leaf traces, as they pass through this layer of vascular tissue, are separated from it by parenchyma. The vascular connections of this peripheral layer with the perimedullary cylinder are never direct but irregular, and of such a nature that both soil minerals and organic foods may pass through a number of different routes to their destination. The bundles of the stem are for the most part amphivasal, with a layer or two of parenchyma separating the xylem and the phloem (fig. 27*B*). The amount of xylem on the side of the leaf trace farthest from the pith diminishes as the trace extends upward and outward, so that in the leaf the bundle is collateral.

A rather thick parenchymatous cortex penetrated by numerous

roots surrounds the stele. Since successive leaves touch each other, the stem is without an epidermis of its own. In longisection the stem appears somewhat heart-shaped, because successively formed parts have a greater diameter and because the apical meristem is increasing in size less rapidly than the surrounding area (fig. 27).

Adventitious roots

Adventitious roots originate very near the apical meristem of the stem and, because it is slightly sunken, at about the same level as this meristem (fig. 29). Their steles are continuous with that of the stem (fig. 28). Although the pericycle is not distinguishable as a single layer, it is in this region of the stem that the stele of the adventitious root originates. The cortex of the root appears continuous with the inner cortex of the stem. As the root elongates it first pushes through the outer cortical cells, which elongate somewhat during the process, and then through the bases of two or three fleshy sheaths. In so doing occasionally a root may penetrate the inner epidermis of a sheath but fail to pierce the outer. As a result the root usually grows upward for some distance through the mesophyll of the sheath, and finally dies when this becomes desiccated.

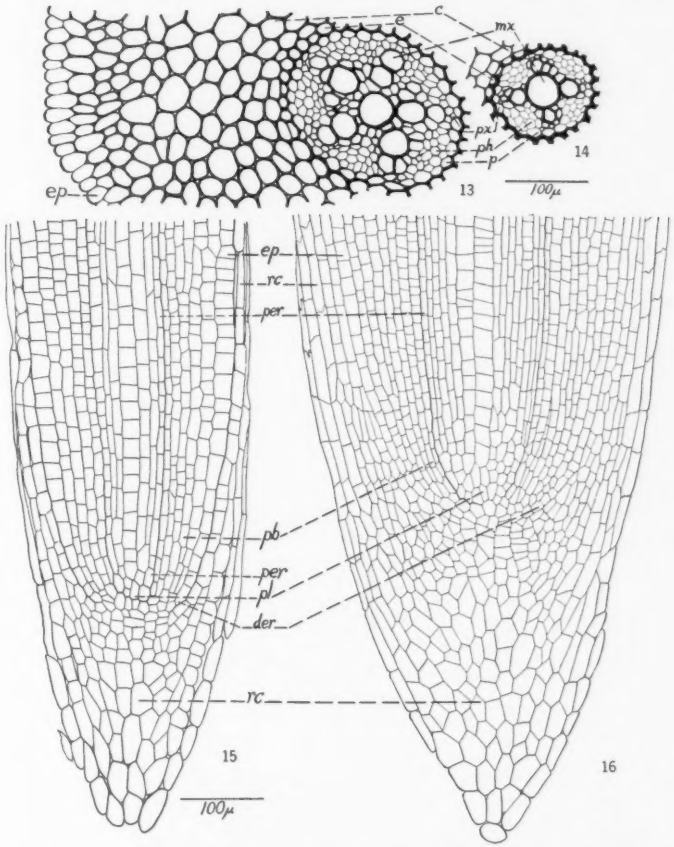
Development of the axis of the adventitious root proceeds exactly as in the primary root, except that in the former each region is larger because of a correspondingly greater number of cells (fig. 16). Tetrarch, pentarch, and hexarch steles occur on the same plant although the pentarch is most common (fig. 13).

In a mature root there is no space between the epidermis of its proximal part and the cortex of the stem through which it has broken (fig. 27). Adventitious roots may branch after they have attained a length of 10–15 cm. These branch roots break through the cortex and epidermis and usually occur on the convex side of the root. Older roots and leaves and older parts of the stem die and shrivel or disintegrate during the first year of a plant's development, thus making the bottom of the stem somewhat flat.

Development of leaf

The phyllotaxy of the onion is 1/2. The apical meristem of the stem from which leaves are formed, and which eventually grows up

into the flower scape, is a low, circular, dome-shaped mass of cells (fig. 18). One side of this region (*c*, figs. 17, 18) grows up more rapidly than the center or axis (*e*) or than the opposite side (*b*), and owing to



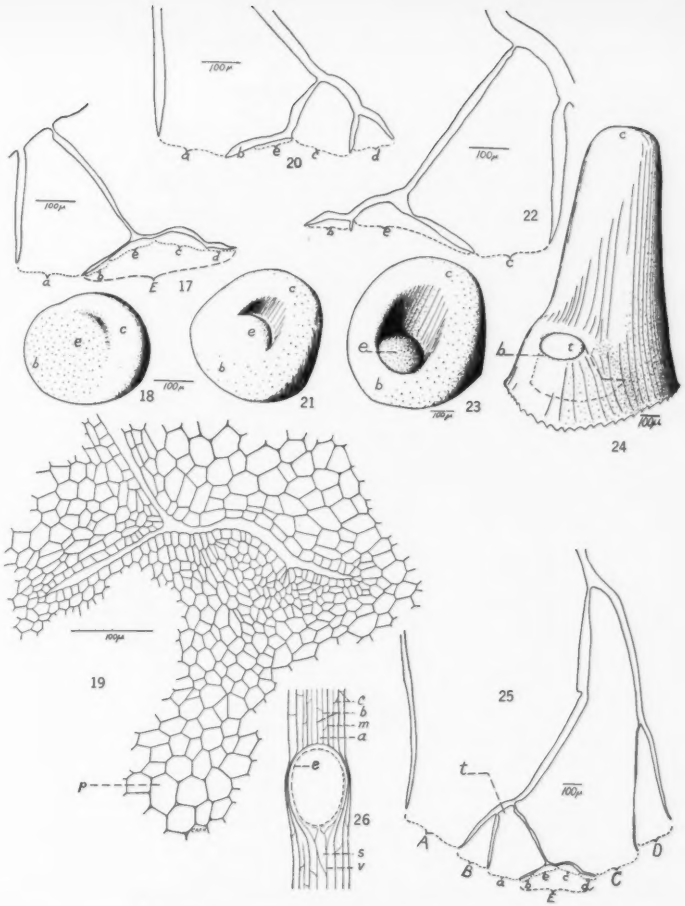
FIGS. 13–16.—Figs. 13, 14, transections of mature portions of adventitious roots: pentarch section (fig. 13) from portion of root near cortical region of stem; tetrarch (fig. 14) from portion 9 mm. distant from stem (*c*, cortical parenchyma; *p*, pericycle; *ph*, phloem; *px*, protoxylem). Fig. 15, median longisection of primary root grown in soil. Fig. 16, same of adventitious root grown in water (*der*, dermatogen; *ep*, epidermis; *pb*, periblem; *per*, pericycle; *pl*, plerome; *rc*, root cap).

centripetal growth as well, soon overgrows the axis (figs. 20–23). As the one side continues to grow higher, the entire periphery of the growing region begins to extend upward around the axis, from which there has now been differentiated the blade of the next leaf, which at this time is also growing at the same rate as the more slowly rising part of the periphery (figs. 24, 25). Thus the vertically oriented, hollow, cylindrical base of each leaf surrounds and incloses within itself the younger leaves and apical meristem. At maturity this cylindrical base of the leaf is the sheath or bulb-scale while the one elongated side is the blade. The rather angular shape of the younger leaves is determined by the surrounding structures, which limit and in a way mold the younger leaves within. During the differentiation into sheath and blade there is much rapid increase in the diameter of the growing region, so that the base of the newly formed leaf is pushed farther and farther away from the center (fig. 27). At the same time the thickness of the leaf blade and of the sheath continues to increase, so that by the time the next younger leaf is differentiated no more periclinal walls are formed. Further increase in the thickness of the sheath to form the fleshy bulb-scale is due to the formation and enlargement of intercellular spaces and to the growth of the cells.

The opening at the upper end of the sheath is designated, for convenience, the orifice of the sheath. A thin membrane extends around the upper edge of the orifice even on the side bearing the blade. Here the membrane is formed by an outgrowth, five or six cells thick, from the edge of the blade, and is usually without vascular tissue (figs. 27, 29).

In the first six or eight leaves elongation is proportionately greater than in later formed ones, and thus this orifice has become longitudinally much stretched so that its sides touch each other and close the cavity within until the next younger leaf is pushed through. In older leaves the tip of the blade is pushed through the orifice while both are inclosed within the sheath of the next older leaf.

In the formation of the first few leaves of a seedling, the blade attains a considerable length before the sheath begins to elongate appreciably. In a newly emerging leaf elongation takes place at about equal rates in all parts. The leaf tip is the first part to cease elongation, however, while the base of the sheath remains meristematic



FIGS. 17–26.—Fig. 17, longisection through stage shown in fig. 18; *a* and *d* are opposite sides of same leaf overgrowing the center (*bec*). Fig. 18, earliest stage of leaf differentiation. Fig. 19, cell detail of region *E* of figs. 17 and 25; plane of cell divisions marks boundary between regions *e* and *c*. Figs. 20, 21, one side (*c*) has outgrown the axis (*e*) and other side (*b*) and is beginning to overgrow it. Figs. 22, 23, both sides (*b* and *c*) overgrowing axis (*e*) which is itself beginning to extend upward. Corresponding parts have the same letters. Figs. 24, 25, the more rapidly growing side (*c*) has developed to form blade of leaf, whereas the upwardly growing periphery forms the sheath or bulb scale. (Small letters of first seven figures correspond to capitals in fig. 25.) *A* and *D*, *C* and *B*, and *a* and *d* are respectively the blade and the sheath sides of successively younger leaves (*p*, pith; *t*, tip of younger leaf). Fig. 26, arrangement of vascular bundles in mature leaf in region of leaf orifice (*a*, adaxial side of blade; *b*, branches from larger bundles; *c*, cross connections between adjacent vascular bundles; *e*, edge of orifice; *m*, bundle continuous in sheath and blade; *s*, outer side of sheath; *v*, bundles ending blindly under orifice).

longer than any other part. Thus in the earliest stage of development the entire leaf is meristematic; at a later date, only the sheath and base of the blade; and still later, only the base of the sheath. The lowest portion of a half-grown blade remains meristematic until the leaf is nearly grown, however, so that this region gives rise to a large share of the blade. Any one leaf, after it reaches a length of 3 cm., attains its mature length in a few days.

Mitosis continues in all parts of the leaf long after intercellular spaces appear (leaf *X* and younger, fig. 27). Transverse divisions as well as growth of the surrounding parenchymatous cells cause these spaces to become enlarged and elongated throughout the entire leaf. At the periphery of the blade in a very early stage of development there is differentiated what appears to be a meristematic layer (fig. 27). As the leaf elongates the cells of this layer do not become elongated longitudinally but radially, so that in the mature leaf they form two or three subepidermal layers of columnar cells corresponding to the palisade cells of dicotyledonous leaves. As these cells divide anticlinally they also increase the diameter of the blade. The inner parenchyma does not keep pace with increase in size, and so a huge central cavity which runs the entire length of the blade is torn through this inner spongy tissue. Before this cavity is formed all the inner cells of the leaf contain functional nuclei, although the volume of the intercellular space is probably greater than that occupied by the cells. After the cavity is torn the cells near its edge soon die, and so by their own collapse, according to NEWCOMBE (**7**), add to the size of the space. The mature leaf therefore consists of this central cavity, surrounded by eight or ten layers of parenchyma with large intercellular spaces, the vascular bundles, the palisade layers, and the epidermis.

Anatomy of leaf

Differentiation of leaf traces begins in the stem adjacent to its xylem, and proceeds upward into the developing leaf. In leaf blades which are no longer than they are thick (fourth younger leaf than *X*, fig. 27), procambial strands are already differentiated. Maturation of the bundles is endarch, beginning in the stem and, once begun in the leaf, proceeding upward rapidly to the tip. The first protoxy-

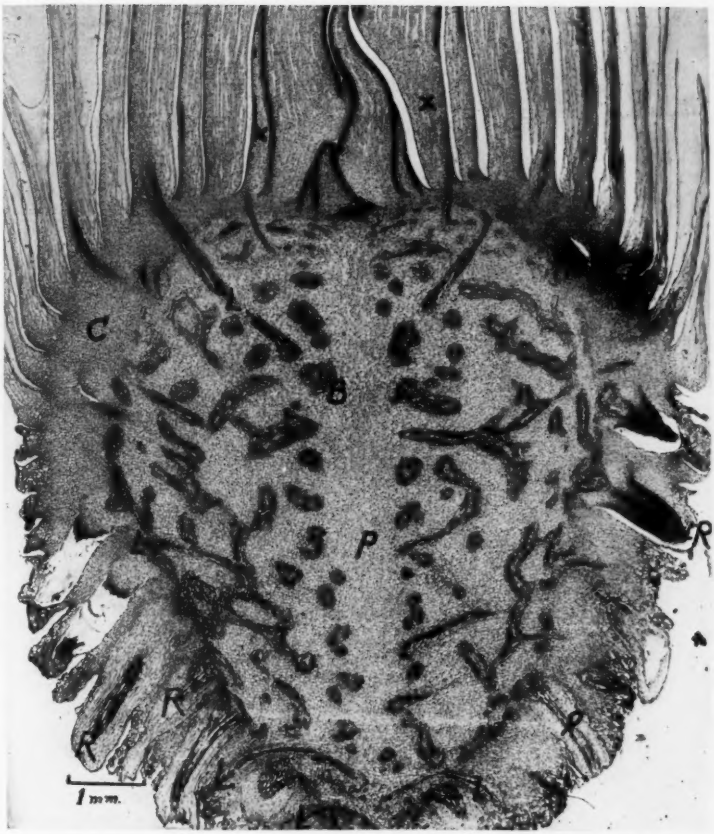
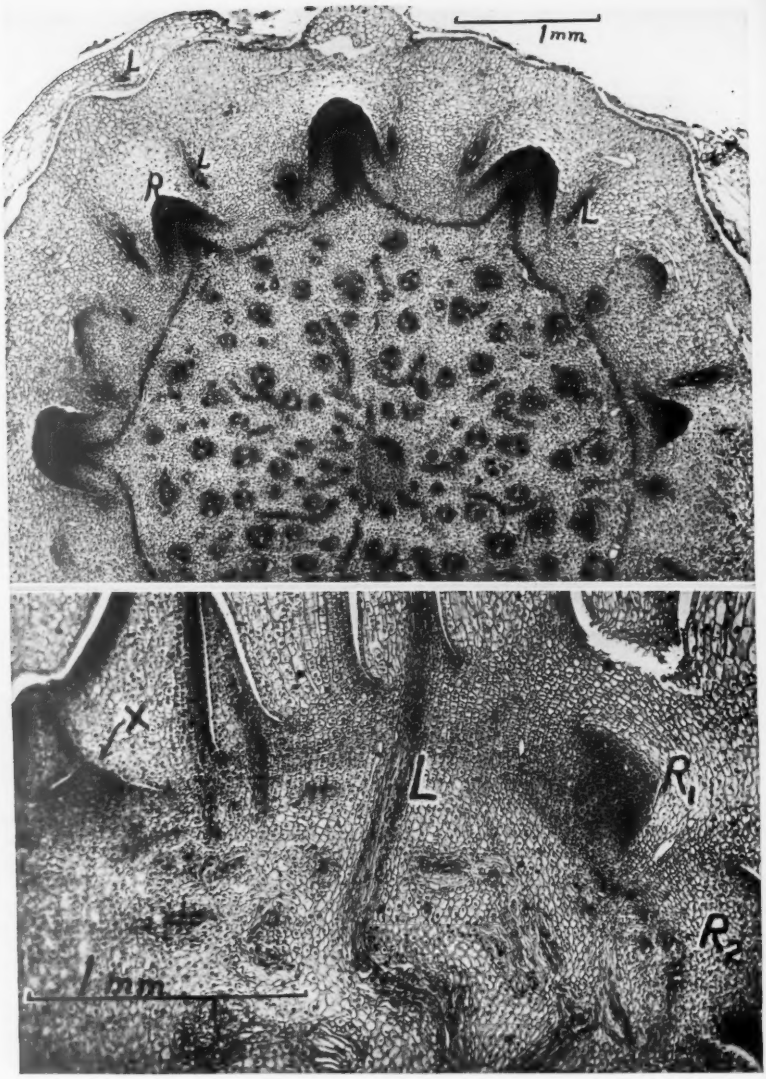


FIG. 27.—Median longisection, except at bottom, through plane of phyllotaxy of stem five months old; bulb development scanty, leaves 40 cm. long, and apical meristem at stage shown in fig. 21. Youngest five leaves contain mitotic figures. Row of dots in outer part of leaf *X* and of next older leaf are transections of connections between parallel leaf bundles. Darker tissue at edges of blades will become palisade tissue (*B*, transection through perimedullary bundle from which bundles of leaf arise directly; *C*, cortex; *P*, pith; *L*, vascular connections of leaf with stem; *R*, same of root with stem; *X*, sheath and blade of one leaf).



FIGS. 28, 29.—Fig. 28 (above), transection of stem five months old 50μ below apical meristem. Procambial strands of four adventitious roots (R) are continuous with outer procambial tissue of stem. Plane of phyllotaxy is parallel to side of page. Bundles shown in transection are arranged in seven concentric circles or arcs, one for each leaf. Of the innermost leaves only bundles of blade are shown, bundles of sheath not yet being differentiated. Radiating procambial strands inside of stele connect vascular or procambial tissue of developing roots and of leaves (L, vascular bundles of leaf). Fig. 29 (below), longisection through apical meristem and roots (L, vascular bundle of sheath of leaf; R_1, root primordium; R_2, older root; X, apical meristem).

lem is matured throughout the entire length of the leaf before any metaxylem is differentiated. Practically all of the xylem consists of spiral elements.

The cotyledon has only one vascular bundle; the second leaf has five; the next has usually eight; and successively older leaves may have one or two more bundles than the preceding leaf, until in the sheath of a mature bulb there are often as many as forty. These bundles are arranged around the periphery, run parallel throughout its entire length, and usually retain their individuality in spite of rather frequent small branchings and cross connections. These cross connections originate in one or several rows of parenchymatous cells which lie adjacent to and between the bundles. The cells divide at various angles periclinally and transversely to form many smaller cells which mature as a cross connection (fig. 27).

In both sheath and blade smaller and larger bundles may originate blindly or their bases may be anastomosed with larger bundles. Some of the smaller ones end blindly near the base of the blade. In the outer sheaths of a mature bulb there are very few cross connections between adjacent vascular bundles in the lower half, but many in the upper. There are cross connections at all levels in the unthickened sheath of a young onion. In outer sheaths of bulbs the vascular bundles often appear green because here a layer of subepidermal chlorenchyma almost surrounds them.

In a mature leaf the circular orifice of a young sheath has become oval and appears laterally placed, owing to a greater elongation of the sheath on the side bearing the blade. Near the base of the sheath vascular bundles are rather uniformly distributed, but near its top the bundles are more closely crowded together on the side from which the blade develops than on the opposite side. In the middle of this latter side are one or two smaller bundles which, after several branchings and cross connections, end blindly under or around the upper margin of the sheath. On each side of these is a larger bundle which parallels them up to the orifice and then is curved so as to parallel its edge. At the base of the blade these two bundles are again vertically oriented, making a sharp angle with their former course around the edge of the orifice. From this angle a smaller branch is continued around the periphery of the orifice

until, near the middle of the base of the blade, it meets a like branch from the other side. At the junction of these two a vertical bundle extends upward into the blade. Between this vertical bundle and each of the original large bundles is usually one other bundle extending upward from the branch around the top of the orifice. Frequently also the original large bundles have a vertical branch extending into the blade before they themselves become vertically reoriented. On either side of these two main bundles, the other bundles of the sheath are crowded together more closely near the sides of the orifice, but again acquire a uniform peripheral distribution in the blade (fig. 26).

In the cotyledon and in all subsequent leaves there occur longitudinal rows of elongated cells situated usually two cell layers beneath the epidermis. These are rows of lactiferous cells which are filled with a whitish fluid containing, according to RENDLE (9), resins and a substance easily hydrolyzed to allyl sulphide. It is this milky liquid which gives the broken onion tissue its characteristic taste and smell. The lactiferous cells in a longisection of a cotyledon 14 days old are easily recognizable, since they stain differently from the surrounding cells, are several times longer, and contain proportionately longer nuclei. In older leaves they occur just within the chlorenchymatous layers also. Their position has no relation to that of the vascular bundles. RENDLE believes that they probably perform either an excretory or a protective function, or both.

Stomata are numerous on the cotyledon of a seedling less than a week old. An epidermal cell and the two guard cells at one end of it were originally one cell. In the development of the stoma, this one cell, instead of dividing near its center as previously, divides at one end, cutting off a characteristically long epidermal cell and a square cell. This cell by one longitudinal division forms two cells which, as soon as the stoma between them is formed, become its guard cells. In a mature leaf the guard cells are sunken beneath the remainder of the leaf surface by more than their own thickness and the adjacent epidermal cells have overgrown them. The cuticle on the exposed epidermal cells is half as thick as the diameter of their own lumen. The surface of the leaf is glabrous and slightly glaucous.

Bulb

The onion bulb consists of a very short stem bearing adventitious roots and a series of thickened leaves and leaf sheaths, the older surrounding the younger, which have at their center still younger leaves and usually several apical meristems. The blades of the leaves may be functional, dried down, or, in the older of the inner leaves, undeveloped. In a large mature bulb the functional blades of the outer six or seven leaves have become dried and broken off, leaving only the sheaths. The outer of these sheaths have themselves also become dried and form a protection for the fleshy storage leaves within. Inside are three or four leaves with very short undeveloped but usually green blades, and inside these are successively younger leaves with blades proportionately longer as the leaves are younger and smaller. In the spring it is these youngest leaves that continue development and become green. The storage leaves outside of them also elongate, especially their sheaths, but their green blades remain relatively small. As the inner and younger leaves grow, the outer fleshy storage leaves become progressively dry from the top of the sheath to its base, because of withdrawal of stored food and moisture. Before leaf growth occurs, however, numerous roots are developed from the periphery of the stem. These extend into the soil and absorb moisture for this whole process.

The time at which formation of the bulb may be initiated varies. A bulb 1 cm. in diameter may be formed when only the fourth leaf shows, or eight or ten leaves may be formed before there is any evidence of thickening of the sheaths. In the first case the food which is being manufactured is stored, while in the second it is used in the formation of more and larger leaves. These two extremes may occur in plants growing under almost identical conditions.

Summary

1. The structure of the embryo and development of the seedling of *Allium cepa* are described.

2. The primary root is diarch. In its growing point are two histogens, the inner giving rise to the stele and the outer to the remainder of the root.

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Summary

1. The structure of the embryo and development of the seedling of *Allium cepa* are described.

2. The primary root is diarch. In its growing point are two histogens, the inner giving rise to the stele and the outer to the remainder of the root.

3. The adventitious root is usually pentarch, originates in the pericyclic region of the stem at the level of the apical meristem, and develops in a manner similar to that of the primary root. Secondary roots may be developed from the adventitious roots.

4. The first vascular tissue to mature is in the cotyledon. Xylem matures centripetally throughout this structure.

5. Transition from an exarch to an endarch condition of the xylem in the lower part of the stem is described.

6. The stem consists of a parenchymatous cortex, a stele which contains branched and anastomosed amphivasal vascular bundles in a groundwork of parenchyma, and a central pith surrounded by a cylindrical network of bundles to which most of the leaf traces are connected.

7. The development of the leaf is described. Vascular bundles of the leaf parallel each other, connect at frequent intervals, and are situated near the periphery of the leaf. The formation of guard cells and stomata is described.

8. Longitudinal rows of lactiferous cells are near the surface of the leaf but without relation to the vascular bundles. These rows never form vessels.

9. The structure of an onion bulb is described.

The writer gratefully acknowledges the valuable assistance and helpful criticism of Dr. H. E. HAYWARD during the progress of this work. For the pedigreed seeds used in growing the material for this study he is indebted to Dr. H. A. JONES of Davis, California.

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CHLOROPHYLL CONTENT OF GRAIN SORGHUMS

J. C. Ireland and Parks A. Yeats

(with plate vii and three figures)

Introduction

In conducting an inheritance study in sorghum hybrids, it was observed that several selections showed pronounced chlorophyll deficiencies after four seasons of self-fertilization. Similar variations have been described by Conner and Karper (1), and our chlorophyll deficiencies seem to be included in their classification. There was some question as to whether the selections showing these deficiencies during the seedling stage would show any pronounced differences in yield or in later development.

The plan has been to make quantitative estimations of the chlorophyll at regular intervals during the growing season. A multiple correlation study of the parts of kafir plants was started about four years ago, and it was decided to include the chlorophyll content as a possible factor contributing to yields. Soil temperature, light intensity, and evaporation records have been made as a part of the experiment station routine. The correlation studies were of some value in determining the importance of physiological factors involved in chlorophyll deficiencies.

A study was made of the daily and seasonal changes in the chlorophyll content of grasses in South Africa by Henrici (4). She showed that there was a pronounced difference between the quantity present in the morning and that in the afternoon. This daily variation was influenced by the amount of moisture present. Following a heavy rain there was an increase in chlorophyll content, and continued drought caused a decrease in the content. High temperatures at night also caused an increase. Her estimations were made twice daily by extracting with acetone and separating with ether. Comparisons were made with a standard copper chlorophyll obtained from Stoll to determine the amount.

A later study of seedlings has been made, in which the plan of

LUBIMENKO and HUBBENET (6) has been followed. The microspectro-colorimeter replaced the photoelectric-colorimeter in order to make more direct comparisons and to eliminate errors. Decomposition of the chlorophyll and fatigue of the photoelectric cell were the most noticeable sources of error.

Methods

PLANT MATERIALS.—The variety of kafir employed was a cross of Blackhull White with Japonica. The plants were arranged in 22 rows, and were grown from selfed seed which had been carried for four generations for a multiple correlation study. The work was begun during the last week of June, and samples were taken from the rows each week of the growing season, until the grain was ripe and harvested, at the end of August. Leaves were pulled from the stalks at random and placed in a paper bag bearing the number of the row, collections being taken between 9 and 10 A.M. Two-gm. samples were clipped with scissors from each leaf, weighed immediately, and placed in extraction cups. Ten units of the Underwriters' Laboratories improved form of extraction apparatus were used to make the extraction, and 70 cc. of C.P. acetone was used in each flask. The operation was continued for three hours, and practically all the color was removed from the leaf particles, the acetone in the siphon cups being clear.

CHLOROPHYLL ESTIMATION.—A photoelectric-colorimeter was used to make a comparative estimate of the density of the extract. The glass container was filled with acetone extract and placed in front of the photoelectric cell window, with the galvanometer set so that the reading was zero. The amount of light reduction was recorded directly on the dial of the galvanometer. A second reading was taken by placing a standard green filter inside the lamp house and adjusting the pointer to zero on the dial. These readings were recorded to indicate the difference between a standard green and the acetone extract. This precaution was taken because it is probable that acetone extracted more than pure chlorophyll, and it is possible that the response of the photoelectric cell was not uniform for the different intensities of the medium. The standard solution as prepared by GUTHRIE (2) was used in estimating the amount. His prep-

aration is stable and corresponded favorably to the copper chlorophyll standard. He estimated that it was equivalent to 85 mg. of chlorophyll in a liter of water. In this manner it was possible to estimate the proportional part represented in our extract for the number of milligrams of chlorophyll present in 1 gm. of fresh kafir leaf. Since all of our samples received a uniform treatment, the results are comparable.

Eight additional rows of kafir were used for a second series of determinations. These rows were grown from seed which had shown chlorophyll deficiencies for four generations:

Row no.
145....Black Amber×Kaoliang
147....Black Amber×Durra (showing a 3:1 segregation of green and white seedlings)
150....Black Amber×Durra (showing a 1:1 segregation of green and virescent seedlings)
151....Black Amber×Durra
152....Black Amber×Durra
153....Black Amber×Durra
158....Reed's×Dwarf Br. Kaferita
159....Reed's×Dwarf Br. Kaferita

Duplicate plantings were made in small flower pots, one set being placed in a large earthenware jar and covered with black building paper to insure complete darkness; the others were placed in a well lighted part of the greenhouse. Temperatures of both groups of seedlings were as nearly equal as they could be maintained. After ten days, leaf tips were taken from seedlings, dipped in boiling water, and mounted in water and glycerin for examination with the microspectroscope. Those from the dark chamber were killed before being exposed to light in order to prevent the formation of chlorophyll.

A Zeiss microspectroscope (fig. 1) was used for comparing the leaf colors with a standard solution recommended by GUTHRIE (2) and photographs of the spectra were made for comparisons. (DuPont's 35 mm. panchromatic negative film was used in a Leica camera.) A low-power objective was employed for this examination, the lamps

being adjusted so that the spectra were of equal intensity before the solution and the leaf mounts were placed.

The Zeiss microscope is shown equipped with the tube attachments for solutions, the Zeiss microspectroscope in position, and a Leica film camera for photographing. The latter is supported upon an improvised crane with a tripod head attached to a large burette clamp. The upright is a rachet which may be raised or lowered with-

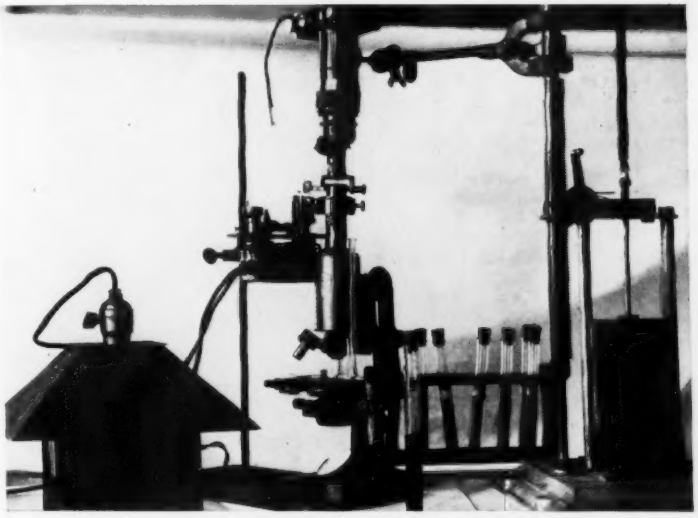

FIG. 1.—Apparatus for microspectral analysis; explanation in text

out throwing the camera out of alignment. In this manner the focus of the microscope may be adjusted and the spectrum observed without delay, and the camera may be quickly swung into place. A bulb control is attached to eliminate any vibration while the shutter is open. The lamps are upon a separate ring stand so that they may be adjusted without delay. Two 6–8-volt bulbs are mounted upon a bell-ringing transformer to provide light for the scale and the standard solution. The covered lamp was used for light through the microscope in order to eliminate any unnecessary light excepting that upon the mirror. The small bulbs are covered with caps when in use

so that their lights shine only upon the openings of the microspectroscope.

Seedlings were allowed to grow for ten days after the examination of leaf tips. Half-gram samples were weighed quickly, ground with washed sand, and treated with 20 cc. of 95 per cent alcohol until the chlorophyll was dissolved. The green extract was filtered into test-tubes. Eight cc. samples were pipetted into the colorimeter tube (fig. 1), the depth of solution in the tube being 8 cm. The apparatus was made according to the directions given by LUBIMENKO and HUBBENET (**6**). A uniform depth for each sample was easily obtained by filling the tubes to the same point upon the scale. Photographs were made of the spectra to show the comparative values of each band (pl. VII). A comparison was made with GUTHRIE'S standard in the small vial attached to the side of the spectroscope.

DATA FOR MULTIPLE CORRELATIONS.—Charts were printed for keeping records of sorghum inheritance studies. Measurements had been made for four seasons of the several characters of the plants, which were considered as indicators of yield. One chart was used for the plants in a single row, averages being taken for each row measurement. The average length of seed branches was taken with a vernier caliper. The lengths of the plants were measured with a surveyor's rod, in feet and tenths of feet. The length of the head and the length of the internode were also noted at the same time. Circumferences of the heads and stalks were measured with a caliper. Counts were made of the number of nodes in the head, the number of side branches, and the number of leaves. The leaf area was estimated by circumscribing the leaf with a Dietzgen planimeter and calculating the area in square inches. This estimation was included in the correlation for 1932. It was used because of the general opinion that the greater the leaf surface, the greater the metabolism and consequently the greater the yield. The galvanometer readings for chlorophyll content represented a convenient range and were incorporated in the correlation of this year. The object of the entire correlation has been to determine mathematically what each character contributed to the yield.

TEMPERATURE AND EVAPORATION.—Records have been kept of soil and air temperatures, together with evaporation readings for the

experiment station. These were considered important in making a study of variations of the chlorophyll content of the leaves. Recording soil and air thermographs were used in making the weekly records of temperature. These were averaged with the planimeter, and the resulting figure was used as a value for that week. Readings of evaporation were made daily with a standard Weather Bureau

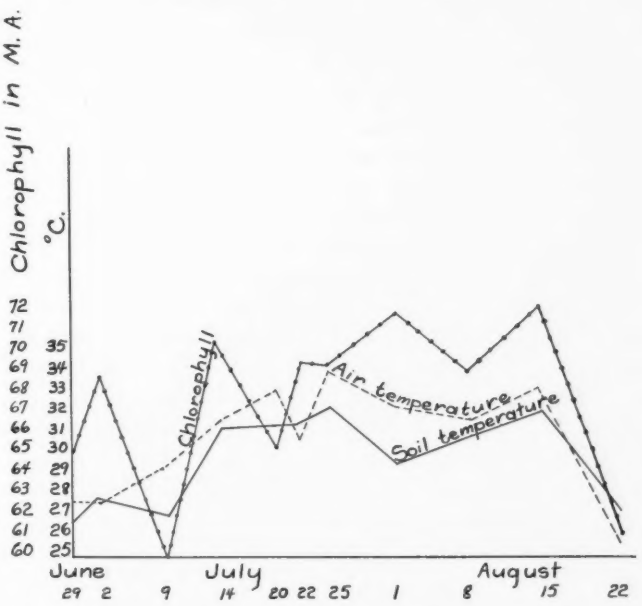

FIG. 2.—Comparison of chlorophyll content with air and soil temperatures

evaporation hook-gage. The averages of these were determined on the days when the chlorophyll content was calculated, so that each reading represents the approximate value for the corresponding periods. Soil temperatures were determined in a plot adjacent to the one in which the kafir was growing, the bulb having been placed vertically, below the surface of the soil. The Centigrade chart (fig. 2) represents a fair estimation of the temperature of the first 18 inches of soil.

Results

CHLOROPHYLL EXTRACTIONS.—Table I gives the detail of galvanometer readings for acetone extracts made during the summer of 1932. There is a noticeable variation in amounts, those of the first day ranging from 56 m.a. to 72 m.a. Records show that most of the rows having a consistently low chlorophyll content had virescent or albino seedlings during the early stages of development. In the rows showing higher averages, the plants had a decidedly darker green foliage and were more vigorous. Plants of the tallest rows (Japonica) do not have the highest chlorophyll content but rather the lowest.

Reading vertically in table I, it may be noted that there is a pronounced irregularity in the determinations for the several periods. HENRICI (4) shows similar deviations, and explains them as due to differences in moisture and temperature. If the differences were due to sudden changes in moisture supply, it would appear that all readings for that date should be low or high in proportion (note the results for August 12). The range is from 23 to 74, with about an equal number of high and low readings. Since the extractions were all made under uniform conditions, it is not probable that errors would cause such a difference.

The milligram weight of chlorophyll shown below the row averages in table I is the estimation based upon the standard used by GUTHRIE (2), in which he states that his solution is equivalent to 85 mg. per liter of water. Since our work was for comparative amounts, for correlation purposes, this estimation is offered as a basis for other work.

Figures 2 and 3 show graphically the trend of the chlorophyll content of leaves as compared with temperatures of soil and air, as well as with evaporation. There is a similarity of these trends, the chlorophyll variations apparently being less pronounced than the evaporation.

A check of experiment station weather records was made to determine whether these increases in chlorophyll were in any way correlated with cloudy or clear weather. There were only four cloudy periods during the entire time, as follows: the last two days in June; the 5th, 6th, and 7th of July; the 27th, 28th, and 29th of July; and the 15th, 16th, and 17th of August. The only observation that might be considered is that clear days following cloudy periods were

TABLE I

PHOTOELECTRIC-COLORIMETER READINGS IN MICROAMPERES OF ACETONE CHLOROPHYLL EXTRACTIONS

Date (1932)	Row numbers, Blackhull White×Japonica																						Average
	195	196	197	198	199	200	201	202	203	204	205	206	208	209	210	211	212	213	214	215	216	217	
June 29	64	63	60	65	61	61	64	67	63	72	62	71	76	44	75	70	68	56	66	67	64	72	65.0
July 2	65	70	68	65	70	69	71	72	69	70	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	69.0
July 9	58	59	60	52	67	55	68	65	68	60	58	58	54	54	62	67	60	58	50	67	54	64	60.0
July 14	68	67	62	60	77	70	79	76	73	74	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	70.6
July 20	65	61	58	52	67	61	68	55	54	67	65	66	71	71	57	69	75	70	62	72	72	77	65.2
July 22	77	75	68	60	69	67	69	70	66	70	70	68	73	73	72	72	70	67	67	73	74	61	69.6
July 25	73	72	74	70	61	67	75	70	67	74	69	73	73	68	71	65	73	64	65	70	71	70	69.8
Aug. 1	62	74	75	74	74	72	74	74	76	74	69	67	72	72	68	72	72	73	67	77	76	75	72.2
Aug. 8	69	69	73	68	71	71	72	70	72	73	65	52	64	69	68	66	70	68	63	78	82	77	69.5
Aug. 15	68	75	68	62	80	73	78	76	74	77	73	.....	71	74	72	75	77	69	72	76	65	70	72.6
Aug. 22	23	67	37	51	66	60	68	67	46	74	62	.....	.....	68	79	74	74	46	64	68	62	58	60.7
Average	63	68.4	64.8	67.2	69.4	66	71.4	69.3	66.2	71.3	65.8	65	69.2	65.9	69.3	70	71	63.4	64	72	68.9	69.3	
Mg. of chlorophyll in 1 gm. of green leaves	3.09	3.34	3.18	3.28	3.4	3.2	3.5	3.4	3.2	3.5	3.2	3.2	3.4	3.2	3.4	3.43	3.48	3.1	3.13	3.5	3.37	3.4	

generally accompanied by increases in the chlorophyll content. Sunlight records, made by a Moll thermopile and recording galvanometer, did not show any pronounced variation in intensity, excepting during the cloudy weather. After the first of August there was a slight decrease in the intensity, but this was gradual and would not induce any pronounced variations in the chlorophyll content of leaves.

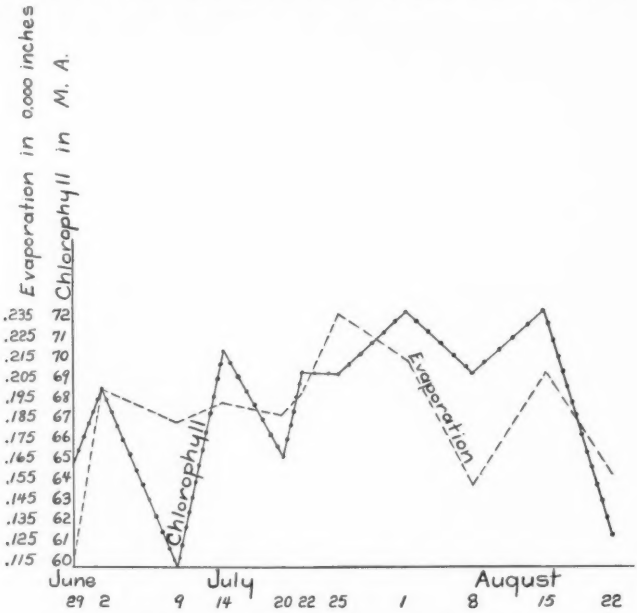

FIG. 3.—Comparison of chlorophyll content with evaporation

The drop in the curve at the close of the period indicates that the plants had reached a stage of maturity and that chlorophyll was not being formed. Practically all the leaves were dead, and the heads were harvested.

MULTIPLE CORRELATION.—The plan of WALLACE and SNEDECOR (7) was followed in the tabulation and solution of the multiple correlation. The array of figures was arranged on ledger paper for convenience in checking the results. In the first division, at the top of the page, the measurements and counts for 22 rows were listed.

The most interesting part of the correlation is the solution of the normal equation (table II). The correlation coefficients are listed in this so that a check may be made of the influences of the independent variables upon the yield. Starting at the bottom of table II, it may be noted that the yield correlates with the chlorophyll content to the extent of 0.535. Compared with the leaf area, the yield has a correlation coefficient of 0.456. The third value from the bottom is also interesting because it shows that the length of the last internode has a −0.572 correlation with the yield, indicating that the higher yields are associated generally with shorter last internodes. Continuing on up to the fourth item, the circumference of the head, it may be seen that it has a 0.859 correlation with the yield. Again the length of the head is negative, as shown in the third row of figures.

The score in percentages at the extreme right of the table is a solution of the Beta values of the correlation. The fractional part that each of these is of the total, gives this percentage. It is a comparative value, but is based upon the entire calculation. The high score of the circumference of the head suggests that it is the most important factor in determining yields. The chlorophyll content is next in value, with 22.94 per cent, and the leaf area is last, with 0.4 per cent. This percentage might be expected to be more, but it has been observed that many small leaved plants produce the greatest amount of grain.

The multiple correlation coefficient of 96.2 per cent is high, compared with those of previous years, and the standard error of estimate is low:

COMPARISON OF MULTIPLE CORRELATIONS (PERCENTAGE)

	1932	1931	1930	1929
Coefficients	96.2	76.2	89.0	74.2
Standard error of estimate	29.0	54.6	64.8	41.6

These comparative figures indicate that the 1932 estimations are considerably more accurate in determining the factors indicating yields. The standard error of estimate of 29 per cent shows that

TABLE II

SUMMARY OF 1932 CORRELATION COEFFICIENT

No.	Variables										Score
	1. Average length of seed branch	2. Height of plant	3. Length of head	4. Circumference of head	5. No. of side branches	6. No. of leaves	7. Length of last internode	8. Leaf area	9. Chlorophyll concentration	10. Yield, the dependent variable	Percentage contributed by each independent variable to yield
1.	1.00	−0.288	0.663	−0.214	0.070	0.016	0.227	0.468	0.281	−0.084	6.95 average length of seed branches
2.		1.00	−0.175	0.153	−0.392	0.371	−0.101	−0.002	−0.359	0.176	7.47 height of plant
3.			1.00	−0.607	−0.151	−0.133	0.347	0.243	0.172	−0.342	8.79 length of head
4.				1.00	0.448	0.263	−0.571	0.305	0.271	0.859	39.09 circumference of head
5.					1.00	0.149	−0.299	0.355	0.489	0.508	1.36 no. of side branches
6.						1.00	−0.117	0.563	−0.195	0.363	11.71 no. of leaves
7.							1.00	−0.297	−0.358	−0.572	1.29 length of last internode
8.								1.00	0.252	0.456	0.40 leaf area
9.									1.00	0.535	22.94 chlorophyll concentration
	Multiple correlation coefficient.....96.2%										100% yield
	Error of estimate.....29.0%										

there is still much to be desired in determining the yield values, but it is a decided improvement over the figures of 1930 and 1931. Table III shows the comparative scores over a 4-year period for the various measurements.

The fluctuations in some measured portions of the plant are of considerable interest. The relative value of the length of seed branches seems to be seasonal, with that in 1929 showing an unusually high score. Environmental variations rather than any trans-

TABLE III

SCORE OF RELATIVE WEIGHTS OF FACTORS INFLUENCING YIELDS

Factors	Percentage			
	1932	1931	1930	1929
1. Average length of seed branches	6.95	13.99	3.0	41.80
2. Length of plant	7.47	18.05	15.9	1.93
3. Length of head	8.79	16.03	13.3	11.93
4. Length of tip branches	0*	4.06	15.7	1.40
5. Circumference of head	39.09	24.52	26.4	24.44
6. Circumference of stalk	0	0.00	0.00	0.00
7. Number of nodes in head	0	1.05	7.8	2.87
8. Number of side branches	1.36	15.89	11.7	0
9. Length of last internode	1.29	6.41	6.2	15.63
10. Number of leaves	11.70	0	0	0
11. Leaf area	0.40	0	0	0
12. Chlorophyll content	22.94	0	0	0
Yields	100	100	100	100

* 0 indicates not determined.

mitted factors may be responsible for these changes. The length and circumference of the stalk have always been low. Because of these low scores, the circumference of the stalk, the number of nodes in the head, and the length of the tip branches were dropped from the 1932 calculation and were replaced by the number of leaves, leaf area, and chlorophyll content. The last is one of the most promising determinations. There may be some doubt as to the mathematical significance of the leaf area; however, physiological theory would naturally place the factor much higher than some of the others.

Spectral comparisons

Plate VII shows the comparative spectra of (1) leaf sections grown in darkness, (2) leaf sections grown in greenhouse light, and (3) al-

coholic solutions of chlorophyll from plants grown in the greenhouse for ten days after the first two samples had been taken.

It may be observed in the first column that there is some variation in the spectra of leaves grown in darkness. Spectrum no. 2 suggests the presence of some green coloring, and it may be due to the presence of chlorophyllogen, as suggested by LUBIMENKO. There seems to be a narrow band in the extreme red portions of the spectra at approximately 700 mμ. These bands in the red are also well marked in those leaves grown in light. Blue and green portions show different results in each selection. Spectrum no. 8 of those grown in daylight is of interest, because it is from leaves of the virescent type having almost no chlorophyll. The third column represents alcoholic solutions from various plantings of seedlings after they had grown to a height of 2–3 inches. No. 13 is a check spectrum of clear alcohol in the glass tube, shown in figure 1. Various widths of bands are observed above or below the 600 mμ mark on the scale. LUBIMENKO and HUBBENET (**6**) found the first bands in etiolated wheat seedlings at 670 mμ to about 620 mμ. Our seedlings were green, with the exception of those indicated "albino" (nos. 9 and 16).

The spectra of GUTHRIE'S solution are shown at the right in each case. Those of the third column are much less pronounced because of a reduction in the time of exposure. The elimination of the portion of the spectrum below the lighter greens is of special interest. To the eye GUTHRIE'S solution is identical with an alcoholic or an acetone solution. A photoelectric-colorimeter will not differentiate, but the microspectro-colorimeter shows a different type of selectivity. It is regretted that we did not have the crystallizable ethyl-chlorophyllide for use in a standard solution.

Determinations of the chlorophyll content of the kafir plants which produced the seed for these studies were made at intervals during the summer of 1932. The values were determined from the acetone extracted material, with the photoelectric-colorimeter.

Discussion

The use of the Underwriter's Laboratories improved form of extraction apparatus to obtain chlorophyll extractions made possible a uniform and rapid means of getting all the coloring matter from the

leaves of kafir employed in this investigation. Acetone may have dissolved other substances in addition to chlorophyll, but the number of samples taken and the extensive period of the project would hardly warrant a complete separation of all pigments.

The comparison of chlorophyll content with temperatures of soil and air and with the evaporation is in accord with the work of LUBIMENKO (5) and HENRICI (4). Higher temperatures suggest higher metabolic processes and consequently more chlorophyll.

TABLE IV

PHOTOELECTRIC-COLORIMETER READINGS

DATE (1932)	ROW NO.								
	145	147	150	151	152	153	158	159	AVERAGE
6/28	70	66	68	61	64	70	72	68	67.4
7/2	56	52	72	54	42	18	70	68	54.0
7/2	63	59	67	52	39	55	65	60	57.5
7/14	65	61	63	64	64	18	66	67	58.5
7/22	58	62	68	68	66	56	71	74	65.4
7/25	61	70	72	68	64	70	70	54	66.1
8/1	74	48	71	72	36	61	70	69	63.4
8/8	65	48	56	69	49	56	70	69	60.2
8/15	70	22	.....	62	30	.....	79	73	56.0
8/22	36	.....	.....	60	.....	.....	63	67	61.5
Average	61.8	54.2	69.5	63.0	50.6	50.6	69.6	67.4	
Mg. of chlorophyll in 1 gm. of leaf	3.03	2.65	3.41	3.09	2.47	2.47	3.41	3.30	

VON GUTTENBERG (3) also confirms this increase and decrease in his carbohydrate determinations in evergreens. The decrease in the amount of chlorophyll with the decrease in evaporation suggests HENRICI'S theory that chlorophyll contents change rapidly, even during the course of the day. The plastid being the basic structure, the chlorophyll seems to be deposited in the network as suggested by LUBIMENKO (5).

Summary

1. The chlorophyll content in kafir, based upon weekly determinations, increases generally to the stage of seed maturity in the plant. It begins to decrease as the grain hardens. The chlorophyll

content varies directly with the amount of evaporation and with the temperature.

2. There is a positive correlation between the chlorophyll content and the yield of kafir.

3. Varietal differences in the chlorophyll content seem to be consistent throughout the growing season.

4. The comparison of seasonal multiple correlation coefficients and their standard errors of estimate has made it possible to reduce the latter appreciably. The elimination of indefinite factors by the scoring process, and the addition of others, have aided in securing a higher correlation coefficient.

5. For comparative purposes, the microspectroscope is very effective in determining the relative concentrations of chlorophyll in kafir seedling leaves. These leaves are of uniform thickness when grown under the same conditions.

6. Solutions may be compared by the use of a microspectrocolorimeter with a greater degree of accuracy than with most colorimeters. Spectral band widths in alcoholic solutions vary according to the intensity of the chlorophyll.

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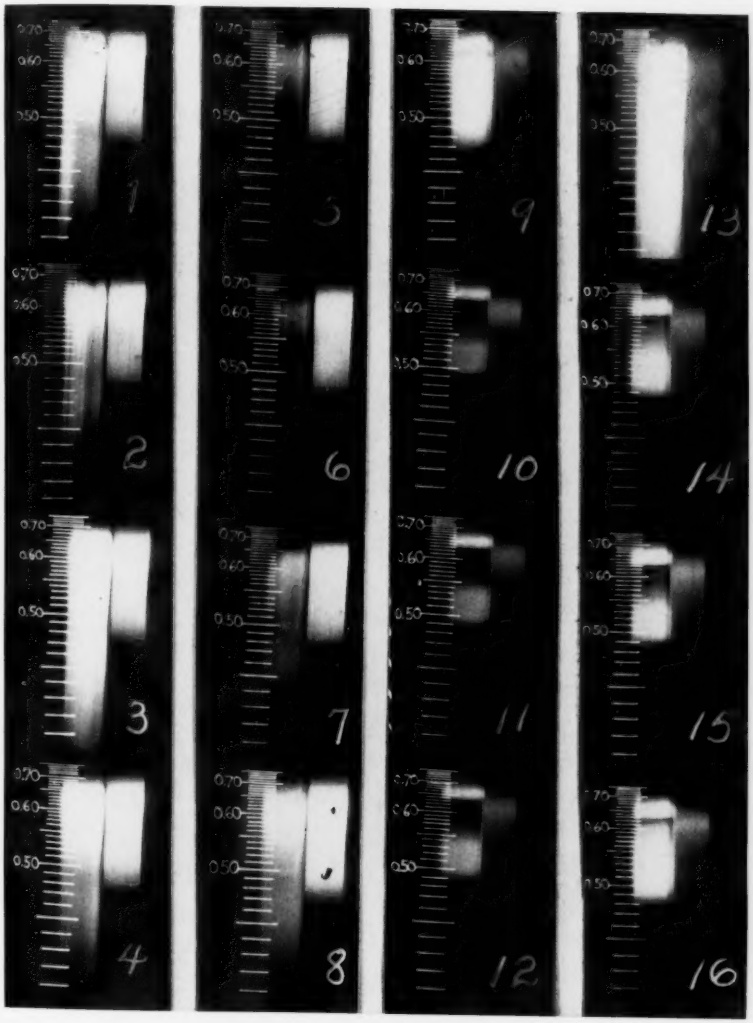

IRELAND & YEATS on SORGHUM

EXPLANATION OF PLATE VII

Comparison of spectra from kafir seedlings.

First column in darkness: 1, from row no. 145; 2, from row no. 147; 3, from row no. 150; 4, from row no. 151.

Second column in greenhouse light: 5, from row no. 145; 6, from row no. 147, green plants; 7, from row no. 147, albino seedlings; 8, from row no. 150, albino seedlings.

Third column, alcoholic solution of leaves: 9, from row no. 150, albino seedlings; 10, from row no. 151, green plants; 11, from row no. 152, green plants; 12, from row no. 153, green plants.

Fourth column, alcoholic solutions: 13, spectrum of alcohol, without chlorophyll; 14, from row no. 145, grown in darkness; 15, from row no. 145, grown in light; 16, from row no. 147, albino seedlings.

ROOT NODULE FORMATION ON THE GARDEN BEAN, STUDIED BY A TECHNIQUE OF TISSUE CULTURE[1]

KEITH H. LEWIS AND ELIZABETH MCCOY

Introduction

Among the fundamental unsolved problems concerning the root nodules of the Leguminosae is the relation of the rhizobia and their hosts before and during the process of nodule formation. The factors which stimulate the bacteria to enter in the first place, the method of their entrance, their complex relation to host during nodule development, and the basis of their specificity for certain host plants (that is, the complete immunity of non-leguminous plants and the well known cross-inoculation grouping within the Leguminosae) are unknown. The interaction of plant and bacteria may indeed begin before the bacteria enter at all, for it is well known that plants exert a certain influence upon the general soil microflora (**14, 6**). It is assumed that the greater multiplication of bacteria in the rhizosphere of plants is due to the greater concentration of organic matter, either from direct root excretion or from the sloughing off of dead cells. WILSON (**20**) and JOSHI (**7**) have further indicated that leguminous plants are in some way particularly favorable to maintenance of the rhizobia in the soil surrounding their roots.

In an early report on the mechanism of infection, it was suggested that leguminous plants through some root excretion exert chemotactic attraction for the rhizobia of the surrounding soil. On the other hand, many have held that chance contact of rhizobia with the numerous root hairs is sufficient to account for infection (FRED, BALDWIN, and MCCOY **5**). THORNTON (**15**), however, has revived the earlier conception on the basis of his observation that the young alfalfa plant, at a stage of development coincident with the opening of the first true leaf, produces a substance which influences infection. Younger seedlings are seemingly immune to infection, although exposed to excessive numbers of active rhizobia. Infection, however,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

may be induced in the young plants by the presence of older plants or of washings from soil about their roots. The stimulating substance therefore appears to be given off from the roots of plants of proper age and to be water-soluble. THORNTON tried to determine the source of the substance by cutting off various parts of the tops, and came to the conclusion that the removal of the cotyledons materially delays the formation of nodules, whereas the removal of the first true leaf or terminal bud has practically no effect. He did not definitely prove the source or nature of the material, therefore, but rather suggested that some general physiological change occurs in the plant, causing liberation of the specific substance at the time of opening of the first true leaf.

That the plant exerts some control over infection is evident in other ways also. THORNTON (**16**) pointed out that there is a definite limitation of the degree of infection, only 4 per cent of the root hairs of alfalfa plants grown in agar being infected even in the presence of excessive numbers of rhizobia. FRED, BALDWIN, and McCOY (**5**) cited a case of secondary setting of nodules upon plants of *Phaseolus vulgaris*, from which the seed pods had been removed before maturity. And DUNHAM and BALDWIN (**3**) found in their experiments with double inoculation that nodule-free plants of a given age were much more readily infected with an effective strain of rhizobia than were parallel plants already bearing nodules of an ineffective strain.

The delicate balance between the rhizobia and their leguminous host is also evidenced by the effect upon the association produced by changing environment. The effects of the various environmental factors have long been studied, primarily as affecting the number, size, and placing of nodules, and effectiveness or resultant benefit to the host. In summary it may be said, ". . . . any factor which tends to promote vigorous plant growth, with the exception of a plentiful supply of combined nitrogen, will enhance the benefit derived by the host" (**5**). In general, the optima for effective nodulation are a plentiful supply of air, moist but not water-logged soil, adequate sunlight, a temperature favorable for plant growth (about 25° C.), neutral soil reaction, and a well balanced nutrient solution rich in the essential elements except nitrogen, which must be low for best setting and functioning of nodules.

Of these several factors only light need be discussed here, since it is the only factor purposely varied in the present work. The depressing effect of inadequate lighting was noted as early as 1888 by VINES (**18**), who reported that *Vicia faba* grown in shade produced no nodules. PRAZMOWSKI (**9**) in 1890 grew pea plants in garden soil for 4 weeks in a darkened room and found nodules as numerous upon them as upon control plants grown in light. They were definitely smaller on the darkened plants, however. Recently LEONARD (**8**) concluded, from a long series of greenhouse tests, that it is chiefly lack of light which causes poor nodulation and poor plant growth during the dark winter months. And since reduction of chlorophyll (by removal of leaves) or insufficient CO_2 supply produces a similar result, it appears that limitation of photosynthesis by any of these means may be responsible for poor nodulation. THORNTON (**17**) also linked the depressed nodulation of his darkened alfalfa plants with lack of photosynthesis. Upon plants kept in complete darkness from germination onward only 3.5 nodules per 20 plants were formed; upon those seedlings darkened after 12 days of growth in light (that is, at the age of opening of the first true leaf), 16 nodules per 20 plants; and upon control plants continuously in the light, 46.5 nodules per 20 plants. It was also noted that upon the second lot of plants, those nodules initiated during the period of light ceased growth after darkening, and that no new nodules were formed. In other words, there appeared to be a direct relation between photosynthesis and development of nodules. WILSON (**21**) demonstrated practically the same point in a different manner. By supplying carbohydrate in the form of dextrose, levulose, or sucrose in 0.5–2 per cent concentration in a mineral salts nutrient agar, he was able to grow plants in complete darkness at 25° C. for as long as 36 days. Under such conditions no nodules were ever formed on alfalfa or clover, but two nodules developed on a pea plant in the presence of 2 per cent dextrose. The vetch plants gave somewhat better results, one nodule being formed even on a control plant without sugar, two on a plant with 2 per cent dextrose, and as many as four on a plant with 0.5 per cent sucrose. Thus in spite of the large number of plants failing to form nodules, it would seem that in the presence of carbohydrate, suitable in kind and concentration, infection and at

least early stages of nodule formation may be induced upon entirely etiolated plants of the Leguminosae.

The experiments to be described in this report have been designed to study further aspects of the complex relation between bacteria and plant, suggested by the work of THORNTON and WILSON. They have therefore taken two directions: (1) the attempt to produce nodules upon excised roots to determine whether there is evidence of the stimulatory root excretion by such (if so, it would necessarily be a localized root product, entirely divorced from any photosynthetic process in the top); and (2) the extension of the WILSON technique to another plant, *Phaseolus vulgaris*, in a different cross-inoculation group. The bean seems a particularly hardy plant for laboratory experimentation and may therefore prove a better host for nodulation than the plants used previously.

Experimental work

The culture of excised roots (that is, of root systems from which the tops have been removed as soon as the roots have completely emerged from the seed coat) is not new. There is in fact a comparatively extensive literature of reports upon growth of dissected plant parts, dating back to 1754 according to a review by ANDRONESCU (1). The most notable recent work on root culture is that of ROBBINS (10, 11) and of ROBBINS and MANEVAL (12, 13). These papers describe a method whereby excised roots of various plants have been kept alive for as much as 12 weeks; they also discuss some of the factors, such as nutrition, light, and aeration, which affect the growth of excised roots.

Preliminary to the experiments of this report, five series of tests were run to explore the possibilities and to find suitable conditions for culture of the excised roots of the desired leguminous plant. Various plants, including clover, pea, alfalfa, lima bean, and wax bean, were tried. The last, locally known as Old's Pencil Pod black wax bean, a variety of *Phaseolus vulgaris* L., was finally chosen for most of the work; the hairy vetch, *Vicia villosa* Roth., was also used in checking these experiments with the result obtained by WILSON with whole etiolated plants. In this early work about 25 variations of four fundamental salt mixtures were used, including the use of

plant extract sterilized by Berkefeld filtration, omission and substitution or removal of nitrogenous compounds from the media, the use of agar, liquid, cellulose, glass bead substrates, etc. Commercial glucose (2–4 per cent) and 400 p.p.m. of autolyzed yeast were in every case added to the media. While no nodules were formed in any of these tests, it was repeatedly noted that the best growth of the roots was always associated with the presence of nitrate. And since this form of nitrogen is particularly depressing to nodulation, many of the variations tried were intended to replace it, but without success so far as nodulation was concerned.

The main experimental work was carried out in two series, the first of which was an attempt to overcome the difficulties encountered in the preliminary tests, and the second to confirm and broaden the suggestive results of the first series. Both were based upon the WILSON technique with modifications, which will be detailed for the first series only.

EXPERIMENT I

The seeds of black wax bean and vetch were sterilized by first washing in distilled water, immersing for 1 minute in 95 per cent alcohol, then for 10 minutes in 1:1000 mercuric chloride, and finally washing 4–5 times in sterile distilled water. The seeds were then allowed to germinate in the dark in petri dishes at 28° C. on yeast water mannitol agar (medium 79 of FRED and WAKSMAN 4). This nutrient agar was intended to favor bacterial and mold growth and thus to facilitate the detection of any non-sterile seeds.

The medium for culture of the plants was the regular WILSON formula:

$MgSO_4$	0.2 gm.
KH_2PO_4	0.2 "
NaCl	1.0 "
$CaSO_4$	0.1 "
Agar	15.0 "
$CaCO_3$	In excess
Water	1 liter

Heat the salt and agar mixture thoroughly to mix and dissolve, add the desired sugar (in this case 0.5 per cent

sucrose), and sterilize in suitable containers (in this case 32 oz. round glass bottles having cotton plugs and containing 250 cc. of the medium). Finally add sterile $CaCl_2$ solution to give a 1:500 molar concentration.

After two days' germination the seedlings were transferred aseptically to the culture bottles. In all, 24 bottles were used for this series, 12 planted to vetch and 12 to beans with 3–5 seedlings in each case. The bottles were then set in closed heavy paper boxes to keep out all light and were placed in a dark 28° C. incubator. The temperature varied actually from 24° to 29° but averaged about 27° C. After two days more of growth the roots were aseptically severed from the tops in seven bottles each of beans and vetch; the tops were not removed from the bottles. The 12 bottles containing beans were then inoculated with 1 cc. of a heavy suspension of *Rhizobium phaseoli* Dangeard, strain 405 of the University of Wisconsin collection, and the 12 bottles containing vetch were similarly inoculated with a suspension of *Rh. leguminosarum* Frank, strain 325. The bottles were immediately returned to the dark incubator and examined only occasionally with artificial light.

The cutting of the roots did not seriously injure either tops or roots, for after a few days the tops sent out strong adventitious roots from the periphery of the cut surfaces; they continued to grow as well as did the plants which were left intact. The excised roots also soon began to stretch over the surface of the agar, and upon reaching the side, turned down between agar and glass. As growth continued the roots branched and rebranched, forming numerous long root hairs which were often visible to the naked eye. In later stages of growth the excised vetch roots began to lag, failed to produce more secondary roots, and became brown and soft. The vetch roots attached to tops grew somewhat better, but they too appeared spindling and discolored and ceased to grow after reaching a length of 10–15 cm. The excised bean roots showed stronger growth, however, often reaching a length of 15 cm. or more and producing 30–50 secondary roots. They were not discolored and appeared to be morphologically normal. The bean roots attached to tops grew even more hardily, as judged by total elongation and number of long and rebranched secondary roots.

Other characters of growth were that all plants were very brittle and had little tensile strength in comparison with sturdy plants grown out of doors; that they were entirely etiolated; and that drying of the exposed roots was not a serious factor in the experiment, owing to the humidity of the incubator and the scheme of incubation in closed boxes.

Sixteen days after inoculation the first macroscopic nodule was observed on the root of a whole vetch plant, and on the 22nd day one on an excised bean root and three on bean roots attached to tops. On the 27th day all of the bottles which appeared to contain

TABLE I

NODULE FORMATION ON EXCISED ROOTS AND ETIOLATED PLANTS OF BEAN AND VETCH; EXPERIMENT I

	BEAN	VETCH
Total number of excised roots	28	28
Total number of plants with adventitious roots only	28	28
Total number of whole plants	20	20
Number of excised roots having macroscopic nodules	1	0
Number of plants with only adventitious roots having macroscopic nodules	2	0
Number of whole plants having macroscopic nodules	4	1
Total number of macroscopic nodules	9	1
Maximum number of nodules per root	2	1

nodulated plants were cut open with an electric glass cutter, the plants removed to pans of water, and their roots carefully washed free from agar. A summary of the nodulation found appears in table I. The small number of macroscopic nodules formed clearly indicates that optimum conditions for nodule formation had not been supplied for either etiolated plants or excised roots. The results, however, do demonstrate that whole etiolated plants of both bean and vetch will produce nodules under the conditions of this experiment; and further that adventitious roots produced upon the stem in absence of the original root system, as well as excised roots of the bean grown in the presence of the tops re-rooted, can also form nodules under these conditions. A detailed study was made of those plants bearing nodules, with the results shown in table II. The

TABLE II

DETAILED OBSERVATIONS ON EXCISED ROOTS AND ETIOLATED PLANTS OF BEAN AND VETCH; EXPERIMENT I

Plant	Number of nodules		Infected root hairs	Condition of root hairs	Condition of root	Length of root (cm.)
	Large	Microscopic				
BEAN						
Top with adventitious roots.....	2	2	+	Very numerous; many curled at tip, bulbed, branched, some stubby	Tips normal; numerous side roots	13
Whole plant..	2	0	−	Numerous; straight, curled, and bulbed; some stubby, others collapsed	Slightly brown, tips normal, many side roots	21
Excised root..	1	1	−	Scarce, short, otherwise normal	Slightly brown, fewer side roots than above, tip of tap root somewhat swollen	9
Whole plant..	1	3	+	Numerous; many stubby, bulbed, curled, or branched	Slightly brown, many side roots	18
Whole plant..	1	2	+	Numerous; curled, some stubby, bulbed; ends rough	Slightly brown, numerous side roots, tips normal	15
Whole plant..	1	2	−	Numerous; long, bulbed, curled	Slightly brown, many side roots, tips normal	19
Top with adventitious root.....	1	1	+	Numerous; long, bulbed, curled	Slightly brown, many side roots, tips normal	19
VETCH						
Whole plant..	1	0	−	Numerous, long, collapsed	Dark brown, few side roots, brittle	

column headed "Number of nodules .... microscopic" refers to those enlargements of the roots that were not readily visible to the naked eye, but which under the low power of the microscope appeared to be very young nodules, distinguishable with fair certainty from young secondary roots. The column "Infected root hairs" is significant only for its positive entries; negative signs mean only that no infected hairs were found.

Free-hand sections of a number of the nodules were made; they were treated with very dilute methylene blue and examined wet under the 4 mm. objective. All of the sections observed appeared morphologically normal, having as the outstanding characters well developed vascular strands and many large cells in the bacteroid area packed with bacterial bodies. The only detail in which they were found to differ from published descriptions of normal bean nodules (5) was in the absence of stored starch. This is not surprising, since no starch was found by the iodine test in any part of the etiolated plants. Perhaps in the presence of a low concentration of sugar (0.5 per cent) there is no excess absorbed and stored. Another point of interest about the tissues of these nodules was the absence of any sign of that parasitism noted by THORNTON (17) in his darkened nodules. This fact is really indirect support for the hypothesis of THORNTON, that the attack of the bacteria upon the tissues of their host is induced by lack of carbohydrate food. At any rate the present work indicates that bean plants or parts of plants grown entirely in the dark but supplied with proper nutrient solution, lacking nitrogen but including carbohydrate, can produce normal nodules.

EXPERIMENT II

The data of experiment I are obviously insufficient for sound interpretation of results because of the small number of plants in the series, and because of the uncertainty whether the results could be duplicated with any reasonable regularity since the factors controlling nodulation under these artificial conditions are so poorly known. Also the plan of experiment I offered no check on the possibility of nodulation of excised roots alone in a bottle, the excised tops having re-rooted and grown with the excised roots in all cases. In other words, it was not proved in experiment I whether

the excised roots were infected because of the presence of that hypothetical substance originating in the tops, or whether they were self-sufficient in that respect. Experiment II was therefore set up as a repetition of experiment I, with certain additional lots to round out the series. The plan was briefly as follows:

3 whole plants per bottle	20 bottles
3 excised roots (tops removed) per bottle	20 "
3 excised roots plus tops with adventitious roots per bottle	20 "
2 excised roots (tops removed) plus 1 whole plant per bottle	20 "
3 whole plants per bottle[2]	20 "

The seeds, beans only, were sterilized, germinated, and planted as before. Because this series was carried out in midsummer, the temperature usually ranged between 28° and 30°, but at times went as low as 25° or as high as 33° C. In other words, it tended on the average 2° or 3° higher than in the earlier experiment.

Twenty-four days after inoculation the bottles were removed from the dark room and examined. No nodules were found on the 60 whole plants raised in absence of carbohydrate. This is somewhat surprising, because PRAZMOWSKI (**9**), THORNTON (**17**), and WILSON (**21**), whose work has been discussed in the introduction, each observed in rare cases the nodulation of an etiolated plant in the absence of added carbohydrate. It is known that plants vary in their physiological properties, of course, and it may be therefore that the black wax bean is unable to develop nodules without abundant sugar. The nodules in the remaining lots of plants were found upon the surface roots or those between agar and glass; better aeration to these roots or the difficulty of root penetration in 1.5 per cent agar may account for this tendency. Table III gives the results obtained. It appears from the data that the conclusions of experiment I are confirmed, that it is possible under the conditions of the experiment to induce nodulation of whole etiolated bean plants, tops with adventitious roots, or (more rarely) excised roots in the presence

[2] These whole plants were in WILSON'S agar, sugar omitted, to determine whether the presence of carbohydrate was the controlling factor for infection.

of tops re-rooted. And it may be added from experiment II that it is possible, although still more rare, to obtain nodulation of excised

TABLE III

NODULE FORMATION ON EXCISED ROOTS AND ETIOLATED PLANTS OF WAX BEAN; EXPERIMENT II

	EXCISED ROOTS	TOP WITH ADVENTITIOUS ROOTS	WHOLE PLANTS
Total number used	160	60	80
Total number with nodules	6	11	14
Number grown alone	60	.....	60
Number with nodules (grown alone)	1	.....	9
Number grown together (excised roots+tops with adventitious roots)	60	60	.....
Number with nodules (excised roots+tops with adventitious roots)	3	11	.....
Number grown together (excised roots+whole plants)	40	.....	20
Number with nodules (excised roots+whole plants)	2	.....	5

bean roots entirely alone in a bottle. Perhaps these relations will be seen more clearly in a percentage evaluation of the data:

		Percentage of plants showing one or more nodules
Whole plants alone		15.0
Together	Whole plants	25.0
	Excised roots	5.0
Together	Tops with adventitious roots	18.5
	Excised roots	5.0
Excised roots alone		1.7

The maximum number of nodules on any one excised root system was four, on an adventitious root six, and on a whole plant ten. Thus while nodules can be induced to form on excised roots, either alone or in the presence of whole plants or of tops re-rooted, it cannot be claimed that they form as readily as upon whole plants or upon tops with adventitious roots. Also it is apparent, from the comparatively low percentage of whole plants forming nodules in these experiments, that the absence of light interferes with nodule development in some way which the addition of sugar does not entirely overcome.

As to the possible working of a root secretion stimulatory to infection, as proposed by THORNTON (**15**), there is little evidence one way or the other in the present experiment. The fact that nodules did form on an entirely isolated root (indeed four nodules, or more than on any other excised root in the experiment) is positive evidence that infection did occur without any possibility of that hypothetical substance originating in the top. But the case is 1 positive to 59 negative which had the same opportunity. The fact that excised roots in the presence of whole plants or of tops with adventitious roots did present slightly higher positive figures (two in 40 and three in 60 respectively) is perhaps significant, but again the figures involved are too small for conclusive results. Furthermore, the fact that nodules form less readily on excised roots than on roots attached to tops is not necessarily proof that normally the roots derive from the tops some substance beneficial to nodulation. The alternative explanation, that in the excised root there exists some unrecognized pathological condition, is quite as defensible. It is true that the excised root, as just stated, appears to be morphologically whole, but of its physiological normality we have no knowledge. Growth is our only criterion, and that is admittedly depressed under the conditions of these experiments.

Summary

1. Excised roots and etiolated plants of the black wax bean, a variety of *Phaseolus vulgaris* L., have been raised in artificial culture by an adaptation of the WILSON technique. Growth of the roots is admittedly not normal, in amount at least, as compared with the standard for root development of field plants. (WEAVER and BRUNER [**19**] state with reference to beans: "Plants only in the cotyledon stage, but grown in warm, mellow soil, often have well-branched tap roots twelve inches long.") In the present experiments the roots usually attained about half that length, occasionally two-thirds. They were also more brittle than normal but no regular morphological abnormality could be found.

2. Nodulation (*a*) of excised roots (grown alone or in combination with etiolated whole plants or tops with adventitious roots), (*b*) of etiolated tops having adventitious roots only, and (*c*) of etiolated whole plants of the bean has been accomplished.

3. The ratio of nodulated roots of the various types to the corresponding non-nodulated is low, and indicates that some factor, as yet unknown, is depressing nodule development. It appears further that sugar is essential to nodulation in these experiments but does not entirely overcome the effect of absence of light. The excised roots are distinctly less susceptible to infection and development of nodules than are the roots of etiolated whole plants or of tops re-rooted.

4. The nodules studied were histologically normal but lacked the usual starch of the bean nodule. Possibly this lack indicates merely that no excess was absorbed and stored from the 0.5 per cent sucrose in the nutrient. There was no sign of that parasitism noted by THORNTON (17) and attributed to lack of carbohydrate food in his darkened nodules.

5. Study of the results of these experiments for their bearing on the question of a root secretion stimulatory to infection shows little either way. Nodulation of entirely isolated excised roots is positive proof of infection unaided by any hypothetical substance originating in the tops, as late as that reported by THORNTON at least. The increase in nodulation of excised roots grown with whole plants is slight and certainly insufficient to indicate the working of a root secretion. The considerably increased nodulation of etiolated whole plants or tops re-rooted might suggest the presence of a substance derived from the tops and favorable to nodulation. But there is no guaranty of the normality of the excised root. Some pathological condition, depressing to nodulation, may account for the apparent difference between nodulation of the excised root and of the etiolated whole plant.

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SPORE FORMATION IN SCLERODERMA LYCOPERDOIDES

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(WITH NINETEEN FIGURES)

Introduction

Cytological studies of spore formation in the Gasteromycetes have been few. MAIRE (5) reported that, at the beginning of the prophases of the first nuclear division in the basidium of *Scleroderma vulgare*, the cytoplasm contains a certain number of granules, stained black in iron-alum haematoxylin, from which radiate the microsomes. Two of these granules become centrosomes, acting as the center of radiation. They then become located at opposite sides of the nucleus, whose nucleolus and membrane soon disappear, while the chromatic reticulum is transformed into two irregular knotty clubs which represent two chromosomes and extend almost from one centrosome to another. The spindle is organized between the centrosomes; the two chromosomes contract, then divide longitudinally, and two daughter chromosomes thus pass to each pole. At the pole a chromatic mass covers the centrosomes from which astral rays still radiate. The spindle soon disappears and the second division, which is like the first, begins almost immediately. There is formed around each nucleus a spindle with centrosomes and asters.

RUHLAND (8), PETRI (6), and BAMBEKE (1) agree that the basidium in *Hydnangium carneum* is binucleate at its origin. Fusion of the two nuclei occurs in the spireme stage. PETRI has pointed out that "granulation directrices" separate out from the nuclei and pass to the summit of the basidium. The first division spindle, with a centrosome at each pole, is apical and transverse to the longitudinal axis of the basidium. BAMBEKE found protochromosomes similar to those described by MAIRE, which are replaced by two definite chromosomes at the end of the prophase. The daughter nuclei divide simultaneously. The spindles of the second division are likewise transverse in the basidium. PETRI concluded that a true spindle is

not formed, and that the daughter nuclei are composed of groups of granules without true nucleoli, which are attached to fibers from the base of the sterigmata. According to RUHLAND and BAMBEKE, however, four nuclei with nucleoli and membranes are present at the base of the basidium after the conclusion of the second division. Granules and aster-like radiations are present at the points of origin of the sterigmata. After completion of the second division, the nuclei occupy a clear space in the apical portion of the basidium, become elongated, and each apparently is composed of a mass of granules. RUHLAND and BAMBEKE found that the entire nuclear material of one nucleus streams through the sterigma into the spore, and that a nuclear membrane is formed immediately in the spore. PETRI and ISTVANFFI (4) reported that only a part of the chromatic substance passes through the sterigma into the spore in the form of granules, and that the nucleus is organized from these granules in the spore. In some cases the spore is small while in other cases it is large at the time of passage of the nuclear material. RUHLAND found that one, two, or even three nuclei may pass to one spore, but BAMBEKE reported only one nucleus to a spore. This nucleus later divides.

FRIES (3) found the fusion of the two nuclei in the spireme stage in the basidium of *Nidularia*. Vegetative nuclei measure 1–1.5μ, while the fusion nucleus which practically fills the swollen basidial tip is 5μ in diameter. The fusion nucleus passes through synizesis and later forms a spireme. Longitudinal splitting, segmentation, and contraction of the chromosomes follow. In the prophases the membrane disappears. A long narrow spindle placed transversely to the longitudinal axis of the basidium is formed. Centrosomes are present at the poles. In the anaphase of the first nuclear division, FRIES saw three or four chromosomes at each pole; but at the conclusion of the second division only two chromosomes appear there. After the first division, without reorganization of the nuclei new spindles are formed in an oblique, transverse, or perpendicular position. After the second division, four resting nuclei with membranes are present at the base of the basidium. Granules were seen in the basidium at the base of the sterigmata, and cytoplasmic strands seemed to stream from them to the nuclei. As the spores are formed the nuclei appear to have lost their membranes and to have become

elongated. The chromatic material becomes attenuated and streams through the sterigmata. The nucleus of each spore divides, the mature spores thus being binucleate.

Accounts of the formation of the fruiting bodies of *Scleroderma* and the development of the cavities containing basidia have been given by TULASNE (**10**), SOROKINE (**9**), and RABINOWITSCH (**7**). The young fruiting body consists of a solid undifferentiated mass of tangled, branching hyphae. Although TULASNE thought that various branches of these hyphae form basidia, RABINOWITSCH and SOROKINE found that all the basidia in a cavity arise from the branching of one hyphal knot. Lateral branches become basidia and the surrounding hyphae disappear. Hyphal knots and later basidial cavities appear first in the center of the cavity and later ones develop in a centrifugal order.

MAIRE found the number of spores in *Scleroderma* to be four; TULASNE and RABINOWITSCH found normally four but also variations from two to five; while SOROKINE occasionally found only one spore.

MATERIAL AND METHOD.—The material of *Scleroderma lycoperdoides* Schw. used in this investigation was found on a rotted log in a dense cedar swamp near Douglas Lake, Michigan. The fruiting bodies were dissected from the wood and fixed in formalin-acetic-alcohol and in Flemming's medium solution. Sections were cut at 5μ and stained with Haidenhain's iron-alum haematoxylin or with Flemming's triple stain.

Investigation

The basidia arise as lateral branches of a twisted intertangled hyphal knot as described by SOROKINE and RABINOWITSCH. They are first detected by their darker staining reaction and by their somewhat swollen tips (fig. 1). Basidia of one knot grow toward one point, the knot enlarges, and the hyphae and basidia spread farther apart. The basidia ultimately increase to five times their original size in diameter, and four spores are formed from each. The surrounding hyphae disintegrate, and when the spores are mature the basidia likewise disappear.

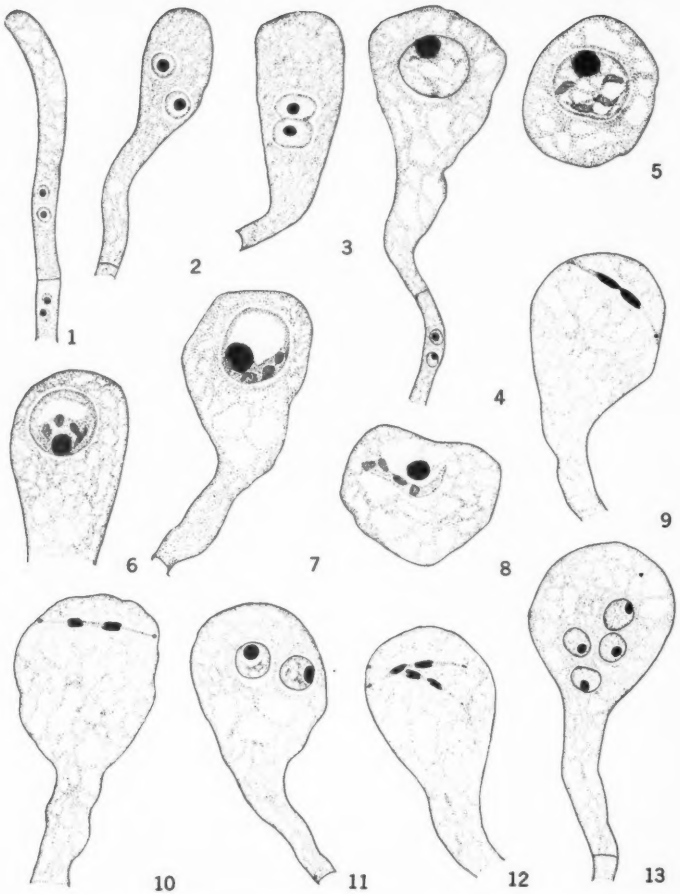
All the cells of the young fruiting body are binucleate. The cell which will become the basidium is 1.6μ in diameter when first recognized, and contains two nuclei which are usually close together.

These nuclei move toward each other and lie in close proximity for a short time. Then they come in contact, become flattened in the plane of contact (fig. 3), the membranes disintegrate, and complete fusion follows. At the time of fusion the basidium is 3.5–4 μ in diameter and the nuclei are approximately three times as large as when first observed.

The fusion nucleus, 2.5–3μ in diameter, is in the apical portion of the swollen basidium. It contains a large nucleolus, around which is a clear space, at least in fixed material. Throughout the rest of the nuclear cavity, however, is a chromatic reticulum in which more darkly stained portions may be distinguished (figs. 4, 5).

In preparation for the division of the fusion nucleus, the chromatic material and denser nucleoplasm become massed toward one side of the nucleus, surrounding the conspicuous nucleolus, which is often pressed against the nuclear membrane (fig. 6). This stage resembles synizesis, characteristic of the heterotypic prophases in higher plants. The synizetic mass tends to spread outward, tapering along the nuclear membrane (fig. 7). In the next stage found the membrane had disappeared, the densely staining mass retaining its shape. Later a long narrow spindle, lying in the apical portion transverse to the longitudinal axis of the basidium (fig. 9), is formed with the chromatic mass at the center. The spindle poles reach the walls of the basidium and at each pole there is a darkly stained body suggestive of a centrosome.

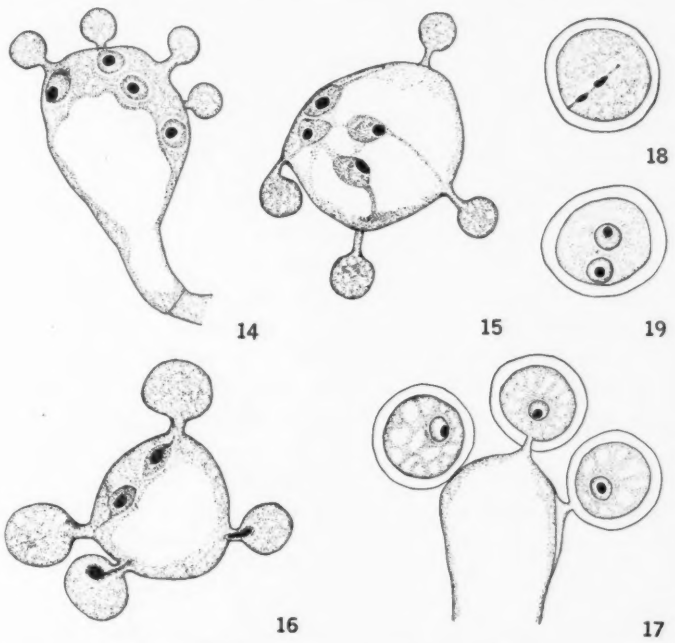
The chromatin mass divides and the halves separate and move toward the poles (fig. 9). In some cases two bodies suggestive of chromosomes may be distinguished in each daughter mass (fig. 10). Two daughter nuclei are organized (fig. 11). The rarity of the two-nucleate as compared with the one- and four-nucleate stages suggests that interkinesis is of short duration. The second division, occurring simultaneously in both daughter nuclei, takes place rapidly. The spindles of this division, like that of the first, lie in the apical portion of the basidium transverse to its longitudinal axis; they make a right or oblique angle with each other (fig. 12). The spindles are in such close proximity that it is often difficult to distinguish one from the other. This division, so far as observed, is similar to the first and results in the organization of four nuclei (fig. 13).



FIGS. 1–13.*—Fig. 1, basidium primordium; fig. 2, young basidium; fig. 3, fusion of nuclei in young basidium; fig. 4, basidium with fusion nucleus; fig. 5, cross-section of basidium and fusion nucleus; fig. 6, nucleus with massed chromatic material; fig. 7, material spread out along nuclear membrane; fig. 8, condition after disappearance of nuclear membrane; fig. 9, spindle of first division; fig. 10, same showing dark bodies at poles and two chromatic masses, each with two parts; fig. 11, two-nucleate basidium; fig. 12, second division; fig. 13, four-nucleate basidium.

* All figures drawn at the level of the table with an Abbé camera lucida, under a 1.25 mm. achromatic Spencer objective on a Leitz microscope with a 20× ocular. Magnification about 3850; reduced to two-thirds.

The four nuclei move to the central or basal part of the basidium, where they remain for a short time. At this stage small narrow protrusions, the sterigmata, are evident at the apical part of the basidium. These outgrowths elongate and at the distal end enlarge and



FIGS. 14–19.—Fig. 14, basidium with sterigmata, the four nuclei pushed to apical portion by vacuole; fig. 15, cross-section of basidium with four nuclei connected to sterigmata by cytoplasmic strands; fig. 16, nuclei passing into distal end of sterigmata; fig. 17, mature basidium showing three of the four spores; fig. 18, division of nucleus in spore; fig. 19, binucleate spore.

become spherical. As soon as the sterigmata begin to protrude a large vacuole is formed below the nuclei; the increase in size of the vacuole seems to cause the nuclei to be pushed to the apical region of the basidium (fig. 14). When the nuclei lie close to the apical wall they become fusiform, their membranes become indistinguishable, and often it is difficult to distinguish the nuclei from one another. A

nucleolus is conspicuous at this time in each nucleus; it is surrounded by a homogeneous group of dark granules. A protoplasmic strand connects each nucleus with the portion of the wall at which a sterigma has been formed (fig. 15). Each nucleus moves toward the corresponding sterigma, becomes greatly elongated, and passes through the sterigma into the enlarged distal end of the latter (fig. 16). In moving through the sterigma, the nucleus is stretched to such a degree that it often becomes threadlike at the lower portion; but as soon as it reaches the distal end it begins to thicken and become spherical. The threadlike portion passing through the sterigma becomes narrower and shorter as the part in the distal end becomes broader in diameter. A wall is formed, cutting off the distal part as a spore. A typical nucleus is soon evident in this spore (fig. 17).

Four spores are typically formed on a basidium and only one nucleus passes to each spore. Later the nucleus in the spore divides so that the mature spore is binucleate (figs. 18, 19).

Nuclear behavior in the formation of the spores of *Scleroderma lycoperdoides* is similar to that described by BAMBEKE (1) for *Hydnangium carneum*, and by FRIES (3) for *Nidularia*, although in *Scleroderma* granules which determined the position of the sterigmata were not seen. The present observations do not agree with those of MAIRE (5) for *Scleroderma vulgare* in regard to the centrosomes. MAIRE found that the second division began immediately upon disappearance of the first spindle, while in the present study binucleate basidia have been found with well organized resting nuclei. These observations suggest that the chromosome number is two, as reported by MAIRE for *S. vulgare*.

Summary

1. Fusion of the two nuclei in the basidium primordium of *Scleroderma lycoperdoides* is followed by enlargement of the basidium and of the fusion nucleus. Two nuclear divisions occur. The four nuclei move to the base of the basidium but later are pushed against the apical wall by a large vacuole.

2. The fusion nucleus passes through a stage suggestive of synizesis. The chromatic material becomes stretched out along a spindle, divides, and reaches the poles. The spindle axis in the apical region is transverse to the longitudinal axis of the basidium. Darkly stained

granules are present at the poles of the spindle, suggesting centrosomes. The second division is similar to the first, and the spindles are close to each other in the apical portion of the basidium. There is indication of the presence of two chromosomes in the second division.

3. The nuclei become elongated, and move toward the sterigma and into its enlarged end. There the nucleus resumes its characteristic form and cell division results in the formation of the spore. Each basidium produces four uninucleate spores. Later the spore nucleus divides.

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ANATOMY OF THE TRANSITION REGION IN GOSSYPIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 449

ALDA MAY SPIETH

(WITH TEN FIGURES)

Introduction

The genus *Gossypium* has been the subject of much investigation from the physiological and anatomical viewpoints. Although much work has been done on the development of the flower and lint fiber, many anatomical details of the vegetative structures require further investigation. This paper describes the root-stem transition region.

Seeds of *Gossypium hirsutum* L. were used in this study, of the upland variety Dixie Triumph. The varieties Acala, Cleveland 54, and Delfos were studied for comparison. The seeds, which were treated with sulphuric acid to limit the development of cotton wilt, germinated rapidly under greenhouse conditions. By the end of the fourth day the hypocotyl may attain a length of 10–12 cm., with a primary root of equal length or longer. In seedlings five days old the cotyledons are fully expanded and the primary tissues are mature in the root and lower part of the hypocotyl. Less mature and older seedlings were used to determine the progressive development and maturation of the tissues.

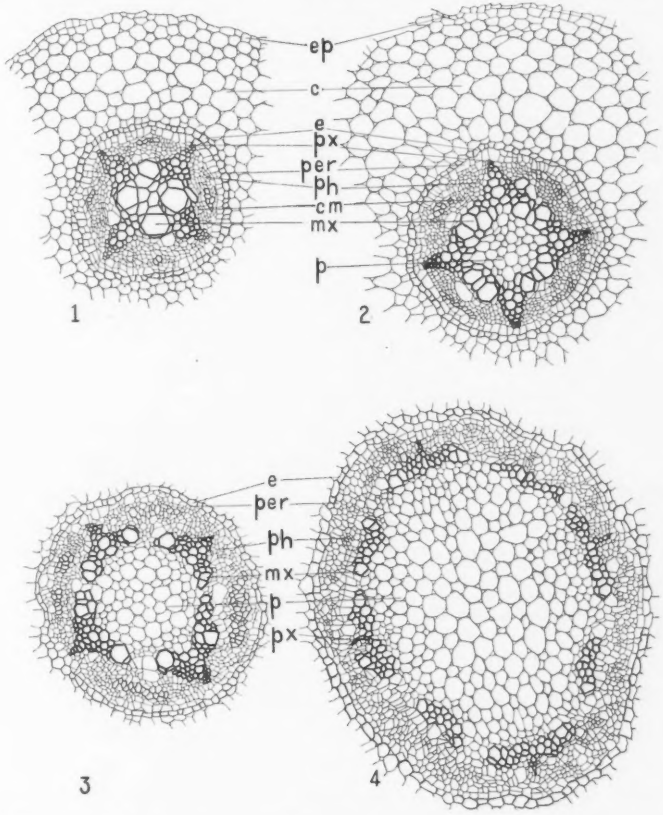
The transition region involves the greater part of the hypocotyl. The xylem is completely endarch approximately 1–2 cm. below the divergence of the cotyledons.

Investigation

PRIMARY ROOT

The primary root of the cotton plant is generally a tetrarch, exarch protostele (fig. 1). The protoxylem is composed of elongated spiral and annular elements, which usually are stretched to such an extent that the thin walls of the annular elements collapse and the spiral bands appear straightened out. The metaxylem adjacent to

the protoxylem consists of scalariform and scalariform-reticulate tracheids; that in the center of the cylinder consists of four or eight large tracheae, which may or may not be separated by cells with



FIGS. 1–4.—Fig. 1, transverse section of primary root: *cm*, cambium; *c*, cortex; *e*, endodermis; *ep*, epidermis; *mx*, metaxylem; *per*, pericycle; *ph*, phloem; *px*, protoxylem. Fig. 2, lower transition region showing pith. Fig. 3, metaxylem separated by parenchyma. Fig. 4, four transition bundles with alternating tangential metaxylem.

scalariform thickenings. The latter elements do not mature until secondary thickening begins.

Alternating with the protoxylem points are four groups of phloem.

These groups are separated from the central metaxylem by parenchymatous cells. The protophloem is parenchymatous and is soon crushed. The metaphloem consists of sieve tubes, companion cells, and parenchyma. When the primary root matures, the walls of some of the phloem cells external to the cambial initials thicken. Thus thickened phloem cells are formed adjacent to the cambial initials. Sieve tubes and companion cells occur at each end of this row of thickened elements.

The pericycle consists of a single layer of cells, external to the phloem region, and of two or three layers of cells over the protoxylem elements. The pericyclic cells are several times the length of their radial diameters.

The endodermis is composed of one layer of cells. The cells abutting on the phloem groups are filled with a mucilaginous substance, but over the protoxylem points this material is usually lacking. The endodermal cells are approximately the same length as the pericyclic cells.

The cortex includes the endodermis and 8–12 concentric layers of parenchymatous cells. These are usually isodiametric, although the two or three rows adjacent to the epidermis may be vertically elongated. The epidermis is a single layer of small cells.

Lateral roots originate early in the development of the primary root. These may be initiated in the pericycle abutting on the protoxylem long before the primary xylem matures.

Root-stem transition

The first indication of a change from the exarch condition of the root to the endarch condition of the stem occurs approximately 1 cm. below the soil level. The hypocotyls that were studied were five days old and were 11–13 cm. in length from the soil level to the point of divergence of the cotyledons. At this age the primary tissues of the lower portion of the hypocotyl are completely differentiated, the cambium layer is active, and secondary thickening is initiated. In the upper portion of the hypocotyl, the primary tissues are beginning to mature. The more meristematic nature of the upper part of the hypocotyl is indicated by the continued elongation of this region and the lack of cambial initials.

At the cotyledonary node there is little differentiation of tissue, indicating that the older part of the axis is the lower portion of the hypocotyl and that the upper portion is much younger.

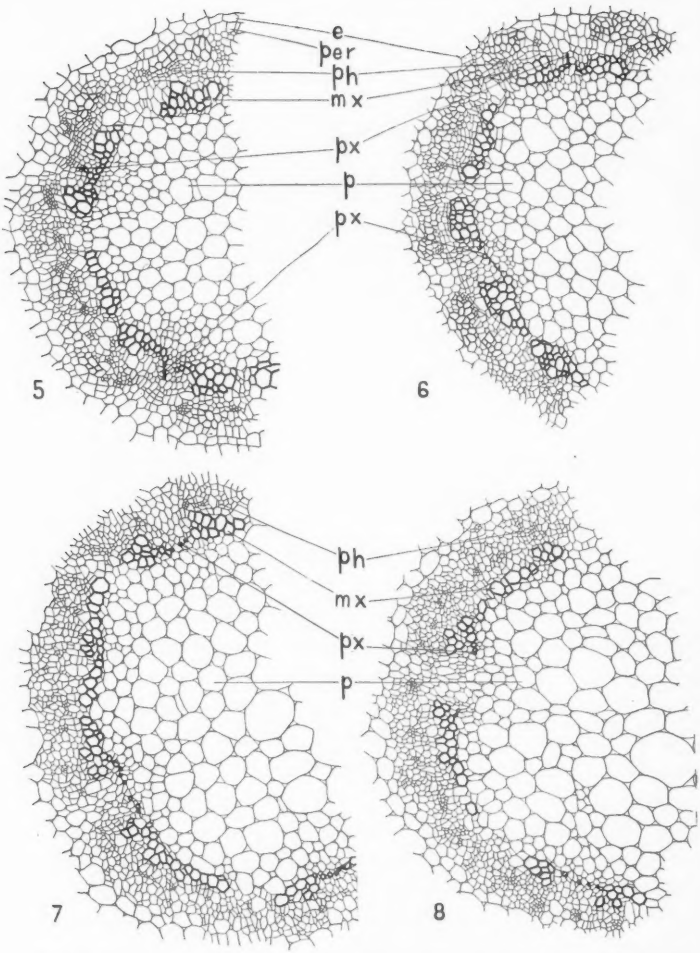
The first change in the root-stem transition takes place where the metaxylem differentiates in a ring, surrounding a pith of parenchymatous cells (fig. 2). The parenchymatous cells of the pith are circular in cross section, with intercellular spaces, and are elongated vertically. The phloem groups are larger, and one or two rows of the cells inside of the pericycle have thickened walls. Sieve tubes and companion cells differentiate at each end of the group, adjacent to the protoxylem points.

Within a short vertical distance the entire stele is enlarged. Four triangular shaped masses of protoxylem and metaxylem cells are laid down (fig. 3). Each triangle consists of the protoxylem at the apex and two tangential arms of metaxylem, which lie opposite the phloem groups and adjacent to the cambial initials.

At a slightly higher level, parenchyma is differentiated between the cells of the metaxylem arms. Four distinct triangular shaped groups of protoxylem-metaxylem elements are formed, with large tracheae at the two inner points of each triangle and with the protoxylem at the apex. Metaxylem continues to differentiate tangentially, so that there are bands of metaxylem alternating with the four transition bundles (fig. 4).

Continuing up the axis, the entire stele becomes larger. The endodermis and the pericycle persist as unbroken bands of cells. The protoxylem points differentiate in the same position, but the triangular shaped masses of metaxylem adjoining each protoxylem point differentiate laterally, so that each row lies at right angles to the protoxylem elements (fig. 5). These four bundles are typical transition bundles. The four large phloem groups are unchanged, except that three or four layers of the cells internal to the pericycle have thickened walls, although the protoplasmic contents are still dense. Small groups of sieve tubes and companion cells may be found at intervals among the thick walled elements.

The next change occurs approximately 2 cm. above the soil level. At this level each of the four original transition bundles consists of two rows of spiral elements separated by parenchyma and two lateral



FIGS. 5–8.—Fig. 5, transverse section of lower hypocotyl showing two transition bundles. Only one bundle shows parenchyma between the protoxylem points. Fig. 6, two transition bundles. In one bundle the protoxylem points are at right angles to metaxylem and are separated by parenchyma; in the second bundle the protoxylem points are in line with the metaxylem elements. Fig. 7, same transition bundles at slightly higher level. Fig. 8, upper transition, showing wide ray between one pair of bundles in contrast to narrow ray of second pair of bundles.

or tangential arms of metaxylem. This condition is first reached in the two opposite bundles. Each of these bundles now consists of two units with the protoxylem in essentially the same position and the metaxylem arranged tangentially. Alternating with these bundles are the other two transition bundles, in which there is as yet no parenchyma between the protoxylem points (fig. 5).

Secondary thickening takes place at this level and the cambium lays down secondary xylem adjacent to the tangential rows of metaxylem. Secondary phloem is differentiated external to the cambial initials. Outside the protoxylem points parenchymatous cells are differentiated by the cambium, and in this way the size of the entire stele is increased.

The endodermis and pericycle retain their identity as continuous bands of cells, but the cortex is composed of 15 to 17 concentric layers of cells. These cells decrease in radial diameter toward the epidermis. At the same level, lysigenous canals may occur in the outer layers of the cortical tissue.

Approximately 4 cm. above the level where transition first begins, the separation of each bundle into two units has been completed. In each unit the protoxylem is still at right angles to the metaxylem. Thick walled pericyclic cells abut on the endodermis, except over the protoxylem points where the pericycle remains parenchymatous (fig. 6). The endodermis appears as a broken band, retaining its identity only over the transition bundles. The cambium remains active and lays down xylem on the inside and phloem on the outside, while over the protoxylem points the secondary tissue remains parenchymatous.

Gradually the protoxylem points of each of the halves of the transition bundles mature opposite each other in line with the row of metaxylem cells (fig. 6). This condition is first noted in two bundles on opposite sides of the axis. In these bundles the protoxylem points are separated by four or five parenchymatous cells. In the bundles alternating with these, the points are more or less at right angles to the metaxylem.

Continuing up the axis, cambial activity decreases and the phloem groups are broken up into smaller units by the maturation of cells as parenchyma (fig. 7). The rays of parenchymatous cells between

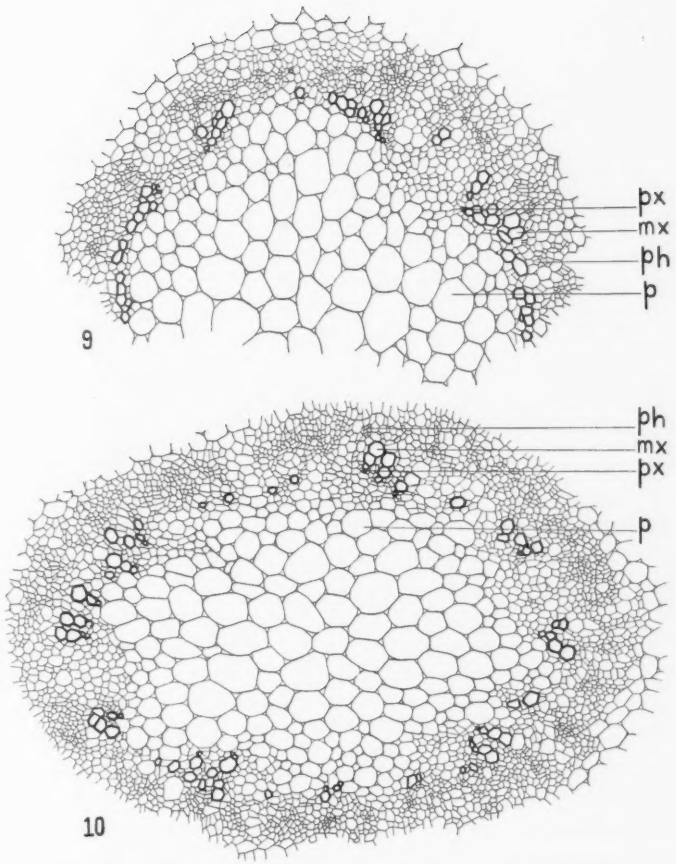
the protoxylem points in the first two bundles to mature increase in size (fig. 8). The pith as a whole remains the same size, but the cells internal to the protoxylem are much smaller in their radial diameters. The cambium at this level is just being differentiated.

The next change is a progressive maturation of the protoxylem inward and a differentiation of the metaxylem outward. Thus by a gradual centripetal differentiation of the protoxylem and a centrifugal differentiation of the metaxylem the endarch condition is reached (fig. 9). This condition occurs first in the opposite bundles. Fewer metaxylem elements mature in each bundle, indicating the more meristematic nature of this region. The parenchymatous rays separating the halves of two bundles become progressively wider, and metaxylem cells appear in the rays. The metaxylem appears progressively, with first one, then two, and finally three cells, each being separated from the other by parenchyma (fig. 9). The rays between the units of the alternate transition bundles remain two or three cells wide. There are no thick walled cells in the phloem at this level, but the sieve tubes and companion cells lie external to the four pairs of transition bundles.

Eventually the endarch condition is reached in all bundles. The rays between the units of the two bundles opposite each other are wide, with two or three endarch bundles in each ray. Alternating with these are the transition bundles in which the halves of each are separated by narrow rays containing no metaxylem elements.

Approximately 8 cm. (in the plants studied) from the soil level, the rays between the units of the two transition bundles that matured first are widened to such an extent that one unit of each bundle is on the opposite side of the axis. Thus four units of the original four transition bundles are on each side of the axis. Each of these groups consists of one original transition bundle, in which the two units are separated by narrow rays, and one unit of each bundle in which the halves are separated by wide rays.

Alternating with the two groups of four bundles are the two or three endarch bundles that differentiated in the wide rays (fig. 10). Each of these bundles consists of a single xylem element and phloem cells. The meristematic character of this level is apparent in the



FIGS. 9, 10.—Fig. 9, two pairs of transition bundles, showing metaxylem element in the wide ray between one of the pairs of bundles. Fig. 10, transverse section of hypocotyl slightly below cotyledonary plate, showing eight endarch bundles. Two pairs of bundles are separated by wide rays, with two or three metaxylem elements in each ray. The other two pairs of bundles are separated by narrow rays.

relatively few metaxylem elements in each bundle and in the undifferentiated character of the phloem region.

At the level of the cotyledonary plate, the four bundles on one side of the axis diverge into one cotyledon. The other four bundles become the vascular traces of the second cotyledon. The remainder of the axis consists of meristematic cells. An examination of the cotyledonary plate of older plants seems to indicate that the endarch bundles, differentiated in the wide rays, become the leaf traces of the axis above the divergence of the cotyledons. The connections of these bundles remain to be determined.

Summary

1. The transition from root to stem in *Gossypium hirsutum* L. is completed in the upper portion of the hypocotyl.

2. The first indication of transition from the tetrarch, exarch, protostelic primary root occurs where the metaxylem differentiates in a ring, surrounding a large pith of parenchymatous cells.

3. At a slightly higher level, parenchyma is differentiated between the metaxylem elements and four triangular shaped groups of protoxylem-metaxylem elements are formed. These are typical transition bundles.

4. Continuing up the axis, the protoxylem elements of each bundle differentiate as two rows of spiral cells, which are formed at right angles to the metaxylem. Eventually these rows are separated by parenchyma. Each transition bundle now consists of two units. At this level a cambium has differentiated secondary xylem and phloem.

5. At a higher level the protoxylem points of two bundles on opposite sides of the axis mature in line with the row of metaxylem elements. Alternating with these bundles are two transition bundles in each of which the protoxylem points are separated by parenchyma, but are at right angles to the metaxylem. Cambial activity has decreased.

6. Farther up the axis there is centripetal differentiation of the protoxylem and centrifugal differentiation of the metaxylem. Ultimately the endarch condition is reached. The rays between the halves of the two bundles that matured first are wide and from one to three metaxylem elements appear in these rays. In the alternate

bundles the rays are narrow with no metaxylem elements. The more meristematic condition of this level is indicated by the relatively few metaxylem elements in each bundle and in the undifferentiated character of the phloem.

7. At the level of the cotyledonary plate, one-half unit of each original transition bundle that matured first, and the two units of the one bundle, in which the rays are narrow, are on one side of the axis. These diverge into one cotyledon. The other bundles become the vascular traces of the second cotyledon.

Grateful acknowledgment is made to Dr. H. E. HAYWARD for his interest in the progress of this study.

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CURRENT LITERATURE

Freshwater algae

The design and production of an advanced textbook has for no group been a more difficult problem than for the algae, owing to the very great number of plant types concerned and to a supposedly limited market. It was solved in England by G. S. WEST in Algae, Vol. I for a general text on morphology and life history, and in British Freshwater Algae for classification with a more limited amount of general information. SMITH has now prepared an excellent text[1] based on the United States flora (to our knowledge of which he has notably contributed) with an extraordinarily full treatment of the morphology and life history of each genus. As the genera are nearly all illustrated, usually by original figures and often in both vegetative and reproductive stages, and as keys to the families and genera are provided, the book is an effective introduction to classification, thus combining the valuable features of its British predecessors. In rare instances the illustrations and text do not seem in full agreement (for example, the figure of *Hormidium* and its generic key character, p. 379). Lacking complete descriptions, and, in all but the smaller genera, citation of more than a few representative species, it is obviously not intended as a taxonomic manual. It would have been of advantage had the author given for each major genus one or more references to fairly comprehensive taxonomic sources, so that the student could quickly reach a just idea of the criteria used in species delimitation. In fact, it will be difficult for the beginner to select from the extensive bibliography that indispensable group of papers without constant access to which he could not expect to accomplish reliable classification of his material. Since the taxonomist must consider that this book is strictly introductory, the reviewer would deprecate the introduction of a considerable number of new names, combinations, and species which to him seem out of place in a general textbook.

Since the approach to the subject is through the American flora, this book is not a general textbook of algal morphology. The morphological material is organized on a systematic basis, and the systematic scheme adopted is conservative. In the case of the Myxophyceae the arrangement is in marked contrast to the most recent general treatment, an elaborate one devised by GEITLER to cover the much more extensive flora with which he dealt. In many groups the student will find novel rearrangements of the material, most of which have good foundation. In the Rhodophyceae it does not seem to the reviewer that placing *Compsopogon* in the Erythrotrichiaceae can be sustained, for against the similarity in method of spore production are great differences in cell structure (a very

[1] SMITH, G. M., The freshwater algae of the United States. pp. xi+716. figs. 449. McGraw-Hill. New York. 1933.

conservative character) and in thallus organization, and the fact that the genus in question is strictly freshwater while its associates in the family proposed would all be marine. Development of the systematics of the Heterokontae, Chrysophyceae, and Chlorophyceae in general enabled the author to incorporate advantageously the rapidly accumulating information on life histories. Separation of the Ulotrichales from the Ulvales by the Oedogoniales would seem to obscure the obvious transition between the first mentioned orders, while the Oedogoniales show no immediate connection with either of the others. Association of the Cryptophyceae with the Dinophyceae in a division Pyrrophyta is accepted, but the Cryptophyceae are omitted as not having an algal type of organization; it would have been advantageous to have developed briefly the characters of the group and its relations with the Dinophyceae, in order that the characteristics of the whole division might have been made clear. Even if their more advanced representatives do not retain algal characters, their common origins with algal types justify at least introductory treatment. The Charales are excluded from the book without explanation, hardly an advisable move in view of the wide belief among phycologists in their algal nature. The great ecological importance of the group makes this lack a serious one, and their physical association with algae encourages their study jointly with other algal types.

These features of organization and content, to which the reviewer has drawn attention, do not seriously detract from the very great value of this text, or its ready usefulness in advanced instruction. It will certainly do much to eliminate ill-informed and superficial study and teaching of algae from American laboratories, and the country is fortunate that its first algal textbook is such a good one.—Wm. Randolph Taylor.

Wild flowers of the Alleghanies

In the eastern United States no region surpasses the Alleghanies in botanical interest. There has recently appeared a charmingly written and scientifically accurate work on the wild flowers of this region. In this book by Harned[2] appear many interesting features, some of which are unique in a book of this sort. Perhaps the most unusual feature is the illustrated key to the families, prepared by Mathews, as are the numerous other excellent illustrations of characteristic species and organs. The eight beautiful colored plates were prepared by Mathews and Eaton. The author designed the volume for the technical botanist and the non-technical flower lover, and has succeeded remarkably well in meeting the needs of these two divergent classes. The specialist will notice the omission of a number of genera and families that would not particularly attract the botanical novice or the non-technical flower lover; among such omitted groups are the grasses, sedges, rushes, pondweeds, willows, and the amentiferous trees.

[2] Harned, J. E., Wild flowers of the Alleghanies. 8vo. pp. xxxii+670. pls. VIII. figs. 400. Published by the author. Oakland, Md. 1931.

In addition to the excellent keys and description of species, the book contains many interesting and important field data, including notes on habitat, color, and agents of pollination. Generally through the volume plant uses are mentioned, especial attention being given to medicinal uses. In many cases the legendary history of interesting species is portrayed. Obviously the author is a field botanist and a nature lover of rare enthusiasm, whose spirit is sure to be imparted to those who read and use the volume. As would be expected, the author is an enthusiastic conservationist, and makes a strong plea for the preservation of our wild flowers. He is to be congratulated on the publication of a work that in content and typography may well be taken as a model of its kind.—H. C. COWLES.

Handbook of plant analysis

For several years the Handbuch der Pflanzenanalyse by G. KLEIN has been going through the press of Julius Springer in Vienna. The earlier volumes have been noted previously in this journal.[3] The final volume[4] has now been published in two large sections paged consecutively.

The first half contains 830 pages, the second 1030. In the first half the methods presented include those for amino acids, amides, amines, betaine, cholin, muscarin, proteins, purines and pyrimidines, nucleins, alkaloids, and cerebrosides. Brief consideration is also given to some little known compounds that include the so-called bitters, etc. The second half takes up enzymes, fermentation methods, antigens and antibodies in plants, analysis for plant hormones, and vitamines. Accompanying the section on enzymes there is a table giving the systematic distribution and occurrence of enzymes in plants. The work closes with a presentation of some special techniques. Biological methods of analysis for certain compounds are presented, such as the digitalis group of alkaloids, aconitin, nicotin, coniin, atropin and other solanaceous alkaloids, physostigmin, morphine, strychnin, caffein, picrotoxin, veratrin, and colchicin. Many of these tests involve the use of frog muscle reactions. Another section is devoted to the analysis of natural waters. Methods for soil analysis, analysis of fermentation media, nitrogen fractionation of plant materials, and the fractionation and purification of pigments by chromatographic adsorption are included among the special methods. An extensive table (pp. 1442–1707) gives certain physical constants for plant constituents. The general formula, melting point, boiling point, solubility, and optical activity are included, as far as they have been determined, for a vast assemblage of known compounds.

The work as a whole is an impressive monument to the industry and patience of the authors, editor, and publishers, who have spent years of effort on it. The user must not expect to find much critical help in the choice of methods to be used with special types of material; nor will he find aid in critical estimation of

[3] BOT. GAZ. **92**:332–333. 1931; **94**:427–428. 1932; **94**:832–833. 1933.

[4] KLEIN, G., Handbuch der Pflanzenanalyse. 8vo. pp. xii+1868. Springer. Vienna. 1933.

the value of the methods. Those who use the work will find it necessary to determine the degree of accuracy, and to consider necessary modifications of methods to take care of precautions to insure accuracy and dependability of the results. It is impossible to have tested the methods critically before compiling them.

The cost of the work, especially at present rates of exchange, makes it prohibitive for the individual. The quoted prices for the entire set in brochure binding is RM 514 (somewhat less than $200) to the American investigator or library. This is significant evidence of the increasing costs of maintaining adequate facilities for the continuation of investigations under our present methods of organization.—C. A. SHULL.

Alpine succession

Plant communities of James Peak, Colorado, have been studied by COX,[5] who, besides shorter studies in other years, spent two entire growing seasons (1928 and 1929) in the field, employing modern ecological methods. COX's station for the observation of temperature was at the lower boundary of the alpine zone, 11,300 feet altitude, while his vegetation studies were carried to the top of the peak, 13,345 feet. The mean annual temperature as well as the temperature for the summer months suggests the Arctic Circle. Frosts occur every month, and most frequently in the higher parts of the zone studied. Floristic censuses were made with 0.5-meter quadrats in meadow and fellfield, and 10-meter quadrats in scrub and timber. The number of quadrats studied in each plant community is so large that the averages of frequency and ground covered must show a high degree of accuracy. An alpine mesophytic meadow climax, with the sedge *Elyna bellardi* dominant, develops in the alpine zone. The successional stages by which this is reached, from bare rocks on the one hand and from pools and wet areas on the other, is carefully traced. Recognized associations and edaphic climaxes include those of fellfield, sedge moor, and meadow with their various minor communities. Species of vascular plants to the number of 197 are recorded from the alpine zone. The chief families with the number of species in each are: Poaceae 21, Cyperaceae 23, Caryophyllaceae 11, Ranunculaceae 8, Brassicaceae 9, Saxifragaceae 9, Rosaceae 10, Scrophulariaceae 10, Carduaceae 28. Since very little detailed ecological work has hitherto been done in alpine regions of America, this study is destined to be rated as a classic to which reference will needs be made in future investigations.—FRANCIS RAMALEY.

Galls

Galls have fascinated both botanists and zoologists since the days of MALPIGHI. The subject matter of cecidology is of great interest to the botanist, not only in its anatomical but also in its physiological, ecological, and pathological aspects. For ecologically minded zoologists, it is of perennial interest because of

[5] COX, C. F., Alpine succession on James Peak, Colorado. Ecological Monographs 3:299–372. 1933.

the symbiotic association of plant and animal. Ross, who has devoted more than 30 years of study to galls, has published the results of his study in an attractive volume which is a signal contribution to the field of cecidology.[6] The volume consists of two parts: a general part of 25 pages devoted to the basic concepts, propositions, and definitions of cecidology; and a special part, devoted to concrete material illustrating the general principles. Twenty-four chapters are devoted to examples of galls selected on the basis of form and origin. Nine chapters are devoted to Cynipid galls. In addition, one chapter is assigned to mycelium-infected insect galls, and an appendix treats the technique of collection and storage of galls as well as culture and identification of gall incitants and the season during which material is collected most advantageously. The volume includes an excellent bibliography and index.—G. K. K. Link.

Cytology

Sharp's textbook on cytology has found such favorable reception in Germany that the second edition has made its appearance as a German translation.[7] The volume is more than a translation; it is a completely reworked, revised edition. The additions and changes made by the translator are marginally indicated. Most changes and departures from the original occur in the discussion of protoplasmic inheritance, influence of genes, mitogenetic rays, the nature of spindle fibers, etc. Three hundred titles of new pertinent literature are added to the excellent bibliography of the second American edition, and the data and concepts of these contributions are integrated into the whole. In the main the translator has carried out a difficult task satisfactorily. The volume is not only a valuable addition to the working library of German botanists, but the additions will render it of service to American and English botanists until a new edition is produced by the author.—J. M. Beal.

Plant geography

A noteworthy contribution to the plant geographic conditions of Mediterranean countries is a recent study of the vegetation of Albania.[8] The author does not merely describe the vegetation of a small country, but also discusses its relation to the Mediterranean and Balkan floras. A large floral map of Albania is included.—A. C. Noé.

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[6] Ross, H., Praktikum der Gallen Kunde. pp. viii+312. figs. 181. Julius Springer. Berlin. 1932.

[7] Sharp, L. W., Einführung in die Zytologie. (Trans. by R. Jaretzky.) pp. iv+733. figs. 212. Bornträger. Berlin. 1931.

[8] Markgraf, F., Pflanzengeographie von Albanien. Ihre Bedeutung für Vegetation und Flora der Mittelmeerländer. Bibliotheca Botanica No. 105. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 132. Illustrated. Stuttgart. 1932. RM 56.